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TREMATODES OF FISHES FROM THE RED SEA

PART 4*—ON SOME NEW AND KNOWN FORMS WITH A SINGLE TESTIS

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MONORCHIDAE

Proctotrema malasi n. sp.
(Fig. 1)

The material upon which this description is based consists of 58 specimens obtained from *Anampses* sp. locally called "Malas." This trematode is small and lanceolate in shape and its size is fairly constant. It measures 0.86 to 1.135 mm in length, and 0.383 to 0.449 mm in maximum breadth about the middle of the body. The cuticle is provided with comparatively thin and long spines. The ventral sucker is slightly smaller than the oral, measuring 0.117 to 0.135 mm in diameter, and is situated slightly anterior to the middle of the body.

FIG. 1. *Proctotrema malasi* n. sp. Ventral view.[1]

The digestive system: The oral sucker is rounded or slightly broader than long, measuring 0.122 to 0.157 mm in diameter. There is a well-developed pharynx which is more or less rounded or slightly longer than broad, measuring 0.068 mm in diameter. There is no pre-pharynx, but a short oesophagus is present. The two simple intestinal caeca do not quite reach the posterior end of the trematode, and are often beaded in their course due to the collapse of the intestinal lumen in some parts.

The male genitalia: The single testis is spheroid, measuring 0.135 to 0.180 mm in diameter and is situated medially between the intestinal caeca at the junction of the middle and posterior thirds of the body. The cirrus sac is long and is bent alongside the anterior and right borders of the ventral sucker which it partly overlaps dorsally. It measures 0.203 to 0.338 mm in length,

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* Part 1—Studies on the family Bucephalidae Poche, 1907. Faculty of Medicine Publication no. 12, Fouad 1st University, Cairo. 1937.

Part 2—The genus *Hamacreadium* Linton, 1910 (Fam. Allocreadiidae). With a description of two new species. The Jl. of the Roy. Egypt. Med. Assoc. 24(7). 1941.

Part 3—On seven new Allocreadiid species. Publications of the Marine Biological Station Ghardaqa (Red Sea) No. 4. Faculty of Science, Fouad 1st. University. 1942.

[1] All figures are camera lucida drawings made by the author.

almost reaching the anterior border of the testis, forming together with the metraterm pouch a large C-shaped structure. It is provided at its distal half with conical spines about 0.013 mm in length.

The female genitalia: The ovary is spheroid, antero-lateral to the testis and in close contact with it towards the right side. It measures 0.68 to 0.9 mm in diameter. No receptaculum seminis could be detected. The vitelline glands occupy a restricted area, more extracaecal in position, roughly between the levels of the posterior borders of the ventral sucker and that of the ovary. The vitelline follicles are more or less rounded, comparatively large, ranging from 8 to 14 in number on each side. There is a congested transverse vitelline duct connecting the two sides of the glands with the oviduct. The uterus reaches the posterior end on both sides of the testis and may occupy practically the whole area posterior to the ventral sucker. Ova are golden-yellowish in color, small, measuring 0.023×0.014 mm in diameter. The metraterm pouch is found anterior and towards the left lateral side of the ventral sucker, measuring 0.158 to 0.225 mm in length. Its distal half is provided with long and slender spines which measure about 0.02 mm in length. A comparatively large genital atrium is situated medially anterior to the union of cirrus sac and metraterm pouch, between the two intestinal caeca. It possesses no spines, a character which refers this species to the genus *Proctotrema*. Sometimes this atrium is displaced from its normal position by being folded back on the distal ends of the cirrus sac and metraterm pouch. It opens to the outside through a common genital pore.

The nearest species to *P. malasi* is *P. minutum* (Manter, 1931) Manter, 1942. The new species differs from Manter's mainly in the following characters: The former species is lanceolate and is more than twice the size of the latter, which is oval. Oral and ventral suckers of the former are about three times the diameter of the corresponding organs of the latter. Intestinal caeca of former extend farther posteriorly than those of the latter. Ova of former much larger than those of latter.

Discussion.—In 1910 Linton described the genus *Genolopa* with *G. ampullacea* as the type and in 1911 Odhner described the genus *Proctotrema* with *P. bacilliovatum* as the type of his genus. Many investigators discussed the identity of those two genera, among whom are Lloyd and Guberlet, Yamaguti, Manter and Hopkins. The latter author in a review of the family MONORCHIDAE (1941), reduced the genus *Proctotrema* to synonymy with *Genolopa*. He found only the usual spination of the cirrus after examining Linton's type specimens of *G. ampullacea*. Manter had previously (1940) considered tentatively that this type species is generically distinct from *Proctotrema* on the basis of two kinds of spines associated with the cirrus sac. In a later publication (1942), the same author, after studying more than 45 specimens of *G. ampullacea* collected by him from eight different hosts, and after comparing these with Linton's type specimens, came to the conclusion that actually the larger spines—previously associated by him with the cirrus sac—are atrial spines usually lying close to the cirrus sac. According to Manter these long atrial spines, which are formed in a semi-circle are very characteristic. In addition, there are two other forms of spines found in the cirrus, and in the metraterm. This triple spination is characteristic for the genus *Genolopa* and distinguishes it from *Proctotrema*.

Yamaguti (1938) created the genus *Proctotrematoides* to accommodate *P. pisodontophidis*. This genus is indistinguishable from the genus *Genolopa*, as has previously been shown by Manter (1942). In 1915 Nicoll described two monorchids, i.e., *Genolopa trifolifer*, in which he could not detect any spines in the cirrus sac or in the vaginal sac; and *G. cacuminata* in which he found the cirrus thickly set with short straight delicate spines, and the vaginal sac without any trace of spines. Those two species therefore, cannot be placed with certainty in any of the genera discussed for lack of sufficient information concerning the spination of the terminal genital ducts. They will therefore be provisionally left in the genus *Genolopa* as originally

proposed by Nicoll, until such time when the type specimens or further material of the two species from the type hosts are obtained and re-examined.

According to Manter *G. elongatum* Manter, 1931 probably should be referred to the genus *Paraproctotrema* Yamaguti, 1934.

The species that have been assigned to the genus *Genolopa* are therefore as follows: *G. ampullacea* Linton, 1910; *G. trifolifer* Nicoll, 1915, *G. cacuminata* Nicoll, 1915, *G. plectorhynchi* (Yamaguti, 1934) n. comb. and *G. pisodontophidis* (Yamaguti, 1938) Manter, 1942.

Those assigned to the genus *Proctotrema* are as follows: *P. bacilliovatum* Odhner, 1911; *P. truncatum* (Linton, 1910) Manter, 1940; *P. lintoni* Manter, 1931; *P. minutum* (Manter, 1931) *Manter,* 1942; *P. macrorchis* Yamaguti, 1934; *P. odhneri* Srivastava, 1939; *P. longicaecum* Manter, 1940; *P. costaricae* Manter, 1940; *P. longovatum* (Hopkins, 1941) Manter, 1942; *P. beauforti* (Hopkins, 1941) Manter, 1942; *P. parvum* Manter, 1942 and *P. malasi* n. sp.

In preparing the following keys to the species of the two genera, the author could not find any means of distinguishing two of the species from their respective similars, i.e., *P. odhneri* from *P. lintoni* and *P. beauforti* from *P. longicaecum.* Therefore the former names are considered as synonyms of the latter.

KEY TO SPECIES OF THE GENUS *Genolopa* (SYN. *Proctotrematoides*)

Species with caeca that do not quite reach the posterior end, ending at about the junction of the 2nd with the last thirds, or the 3rd with the last quarter of the body length:
- Oral sucker smaller than the ventral ..... *G. cacuminata*
- Oral sucker much larger than the ventral ..... *G. plectorhynchi*

Species with caeca reaching the extreme posterior end of body:
- Ovary lobate ..... *G. trifolifer*
- Ovary non-lobate:
  - Vitellaria restricted to area between acetabulum and anterior border of testis ..... *G. ampullacea*
  - Vitellaria more extensive, and reaching to post-testicular area .. *G. pisodontophidis*

KEY TO SPECIES OF THE GENUS *Proctotrema*

Species with caeca terminating at or near the middle of the body:
- Ovary distinctly 3–4 lobate:
  - Eggs ovoid ..... *P. macrorchis*
  - Eggs elongated ..... *P. parvum*
- Ovary entire:
  - Eggs ovoid ..... *P. truncatum*
  - Eggs elongated ..... *P. costaricae*

Species with caeca that do not quite reach the posterior end, ending at about the junction of the second with the last thirds, or the 3rd with the last quarter of the body:
- Oral sucker of about the same size or slightly larger than the ventral:
  - Minute oval form ..... *P. minutum*
  - Larger lanceolate form ..... *P. malasi*
- Oral sucker much larger than the ventral:
  - Eggs elongated:
    - Ovary distinctly lobate and oral sucker is less than twice the diameter of the ventral ..... *P. bacilliovatum*
    - Ovary slightly lobate or entire and oral sucker is more than twice the diameter of the ventral ..... *P. longovatum*
  - Eggs not elongated ..... *P. lintoni* (Syn. *P. odhneri*)

Species with caeca reaching the extreme posterior end of body ..... *P. longicaecum* (Syn. *P. beauforti*)

BIVESICULIDAE

Bivesicula claviformis Yamaguti, 1934
(Fig. 2)

Three specimens, two mature and one immature, were collected from the intestine of *Serranus fasciatus* locally called "Abu Luli." They are small spiny trematodes, 1.3 and 1.7 mm in length and 0.519 and 0.572 mm at their widest diameter about the middle of the body. They are narrowed towards both ends and show a slight dimple-like constriction at one or both lateral sides at about the junction of the middle with the posterior thirds of the body. There is no ventral sucker.

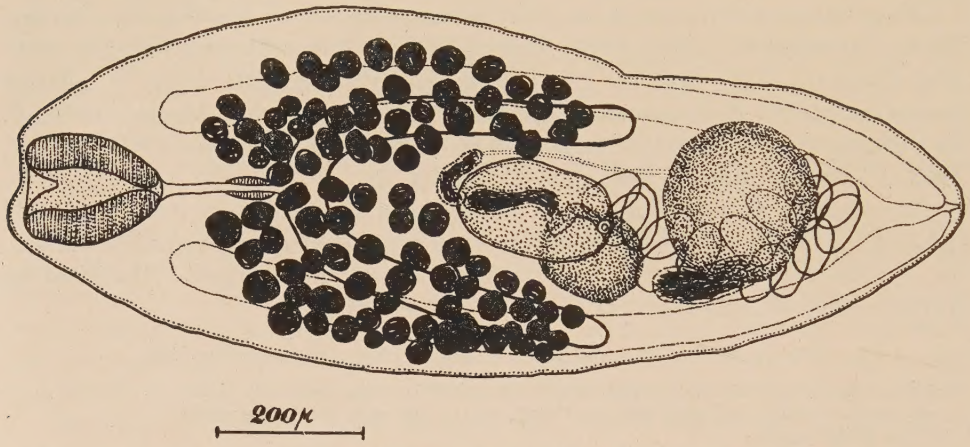

Fig. 2. *Bivesicula claviformis* Yamaguti, 1934. Ventral view.

The alimentary tract begins with a well-developed deeply situated, funnel-shaped oral sucker at the truncated anterior end of the body. It is 0.167 to 0.180 mm in length, and 0.153 to 0.239 mm in width. It leads into a fairly long and narrow oesophagus provided with a weakly developed muscular pharynx. The two intestinal caeca are simple and fairly wide especially towards their posterior ends. They extend posteriorly as far as the posterior border of the ovary, i. e., to about the junction of the middle with the posterior thirds of the body length.

The male genitalia include one testis only which is situated in the median plane in the posterior third of the body, occupying its anterior half. It is a comparatively large, conspicuous, spheroid, smooth contoured body measuring in the two mature specimens examined, 0.203 and 0.261 mm in diameter. There is a well-developed ovoid cirrus sac full of prostate cells. The vesicula seminalis is divided into two elongated parts, an external sausage-shaped one, anterior to the cirrus sac and an internal elongated one. The genital pore is at the posterior pole of the cirrus sac.

The ovary is situated anterior to the testis and to the right side of the median plane in the anterior part of the posterior half of the body. It is spheroid, smooth contoured and measures 0.135 and 0.104 mm in diameter. There is an elongate receptaculum seminis posterior to the ovary, and partly dorsal to the right side of the testis. The vitelline glands are in the form of large spheroid follicles occupying the greater part of the anterior two-thirds of the body, extending from the posterior margin of the oral sucker anteriorly to the level of the ovary posteriorly. The uterus at first extends posteriorly as far as the posterior edge of the testis and towards its right side and then sends another coil back anteriorly close to the above mentioned one. Both coils pass dorsal to the testis, between it and the ovary, to the genital atrium. The ova are few in number, being 8 and 15 in the two mature specimens examined. They are comparatively large in size, measuring 0.081 to 0.09 by 0.05 to 0.086 mm in diameter. They are ovoid, golden-yellowish in color, operculated and possess no shell peculiarities.

The excretory system is composed of two elongated tubular excretory vesicles that extend anteriorly as far as the posterior border of the oral sucker and unite at the extreme posterior end of the body to open in the excretory pore.

This trematode, as all the others of this series of publications, is here described for the first time from fishes of the Red Sea. It is also here recorded from a new host.

Yamaguti (1934) described this trematode from *Seriola quinqueradiata* and *Parapristipoma trilineatum*. He assigned it to a new sub-family BIVESICULINAE, family, MONORCHIDAE. In 1938 the same author described a further two species of this genus, namely *B. synodi* from *Synodus japonicus;* and *B. epinepheli* from *Epinephelus akaara* from Japan. Manter (1947) described *B. hepsetiae* from *Hepsetia stipes* from Florida.

In 1939 Yamaguti erected the family BIVESICULIDAE to accommodate his two genera *Bivesicula* and *Bivesiculoides,* on account of the absence of a ventral sucker and the lack of both the spiny cirrus and metraterm pouch characteristic of all genera of the family MONORCHIDAE.

LEPOCREADIIDAE

Spiritestis arabii n. g., n. sp.
(Figs. 3 & 4 & 5)

The following description is based on six specimens, one of which is immature. They were all obtained from *Mugil* sp. locally called "Boory or Arabi."

The trematode is elongated with more or less parallel edges and measures 1.273 to 2.236 mm in length and 0.338 to 0.678 mm in maximum breadth. The cuticle is provided with minute spines. The ventral sucker is of about the same diameter as the oral or slightly smaller, measuring 0.199 to 0.293 mm. It is situated in a median position, occupying the anterior half of the second quarter of the body length. In Fig. 3 the ventral sucker is displaced towards the right side due to pressure in preparing the specimen.

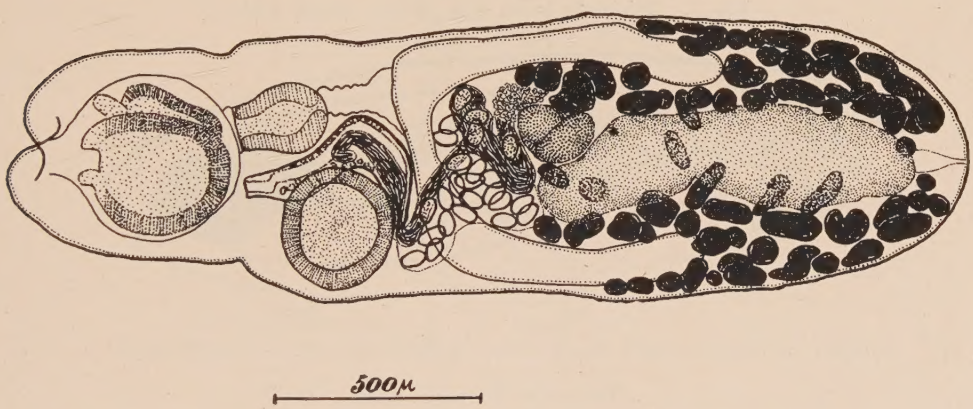

FIG. 3. *Spiritestis arabii* n. g., n. sp. Ventral view.

The digestive system: The oral sucker is roughly pear-shaped, 0.180 to 0.383 mm in diameter, and is provided with four characteristic retractile muscular processes anteriorly. There is no pre-pharynx, but a well developed pyriform muscular pharynx which leads into a short oesophagus. The two intestinal caeca are simple and short, ending at about the junction of the third with the last quarters of the body length.

The male genitalia: There is a single elongated more or less spiral testis, hence the generic name proposed. It is better shown in young forms. It occupies the greater part of the posterior half of the body. The cirrus sac is elongated and is provided with a well-developed seminal vesicle at its proximal half; an elongated vesicula seminalis externa is also present.

The female genitalia: The ovary is more or less ovoid and is situated anterior to the testis. It measures 0.090 to 0.118 mm in diameter. Anterior to the ovary there is a group of cells representing Mehlis' gland. There are two lateral sets of comparatively large vitelline follicles and a few median ones dorsal and ventral to the testis. They occupy the two fields between the testis and the lateral edges of the trematode and extend from the level of the anterior end of testis or slightly farther forward to the posterior end. The uterus occupies a limited space anterior to the ovary, medially between the two intestinal caeca, and opens into a metraterm pouch. A receptaculum seminis uterinum is present. Ova are comparatively large and few,

measuring 0.063 to 0.077 by 0.032 to 0.041 mm. The genital pore is found anterior to the ventral sucker.

The genus *Spiritestis* is characterized by having a length about 3 times the maximum breadth of the body; oral sucker is provided with 4 equal lobes which are retractile; pre-pharynx absent; pharynx well developed and pyriform; oesophagus short; intestinal caeca short, ending blindly posteriorly; single acetabulum about as large as oral sucker; single large testis, elongated antero-posteriorly, spiral, occupying posterior half of body; cirrus sac well developed and provided with a vesicula seminalis interna; a vesicula seminalis externa is also present; comparatively small ovary, entire and spheroid, close to anterior pole of testis; receptaculum seminis uterinum present; vitellaria occupy posterior half of body; uterus contains comparatively few, large ova; is entirely pre-ovarian and opens in a metraterm pouch.

Type species: *S. arabii.*

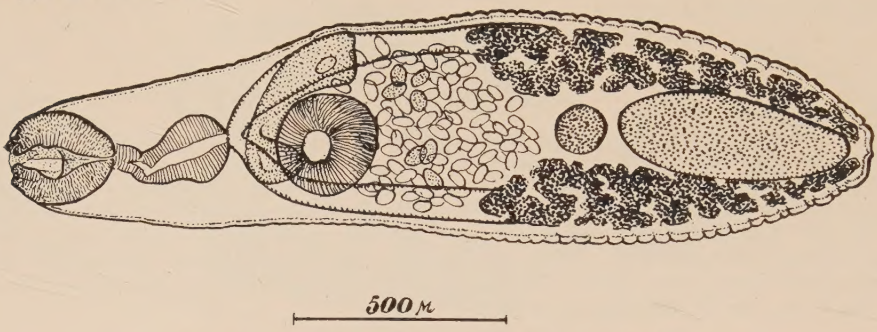

FIG. 4. *Spiritestis arabii* n. g., n. sp. Dorsal view.

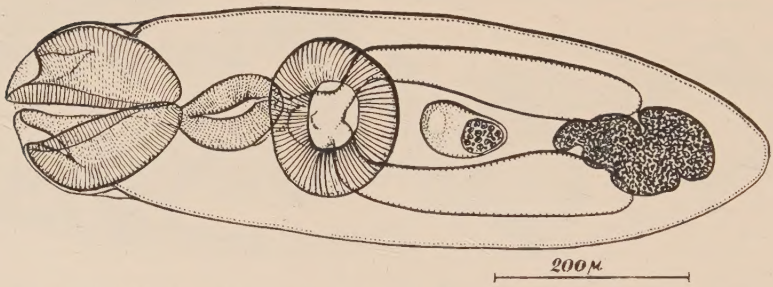

FIG. 5. *Spiritestis arabii* n. g., n. sp. Ventral view of an immature form.

Hairana sohali n. g., n. sp.
(Figs. 6 & 7)

Only one specimen of this trematode was obtained from *Acanthurus sohal* locally called "Sohal." It measures 2.67 mm in length and 0.84 mm in maximum breadth. It is elongate in shape with parallel sides and rounded both anteriorly and posteriorly. The cuticle is beset with spines which are particularly crowded in the anterior part of the body. The ventral sucker is of about the same diameter as the oral and is situated in a median position in the posterior half of the anterior third of the body length. It measures 0.176 and 0.29 mm in diameter antero-posteriorly and transversally respectively.

The digestive system: The oral sucker is situated ventrally at the anterior end of the body and measures 0.220 and 0.282 mm in length and width, respectively. There is a very short pre-pharynx, a well-developed spherical pharynx that measures 0.158 mm in diameter, and is followed by a fairly long, wide oesophagus. This initial part of the alimentary tract is surrounded by many dark-staining glands. The two intestinal caeca bifurcate dorsally to the ventral sucker and are simple, reaching the posterior end of the body. No union and no anus could be detected.

The male genitalia include a single testis situated between the two intestinal caeca at the anterior part of the posterior third of the body length. It is smooth contoured, elongated antero-posteriorly and is more or less pear-shaped. It measures 0.484 mm in length and 0.370 mm in width. A cirrus sac is present and is mainly dorsal and lateral to the ventral sucker. There is an elongated internal as well as an external vesicula seminalis.

The female genitalia: The ovary is a smooth contoured, pear-shaped body anterior to the testis and inter-caecal in position. It is situated at the middle of the body and measures 0.308 and 0.264 mm in diameter antero-posteriorly and transversally, respectively. There is no receptaculum seminis. The uterus occupies a comparatively limited intercaecal space anterior to the ovary and ends in an enlarged metraterm pouch, which opens together with the male genital duct in a common genital pore anterior to the ventral sucker. The uterus contains many ova which are golden-yellowish in color and measure 0.058 by 0.036 mm in diameter.

The excretory system could not be made out in this specimen with the exception of its terminal opening posteriorly.

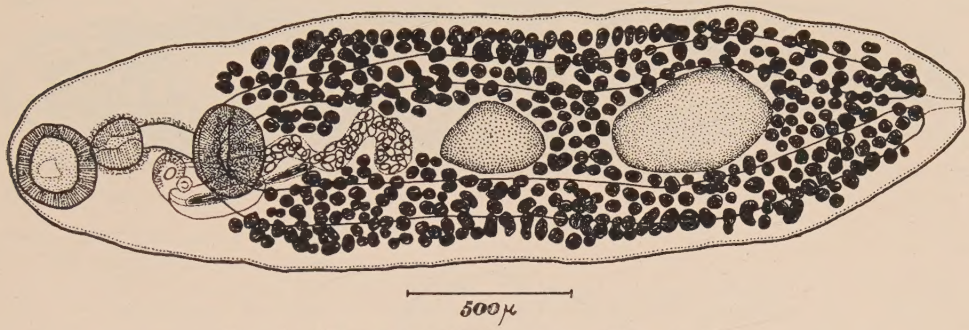

FIG. 6. *Hairana sohali* n. g., n. sp. Ventral view.

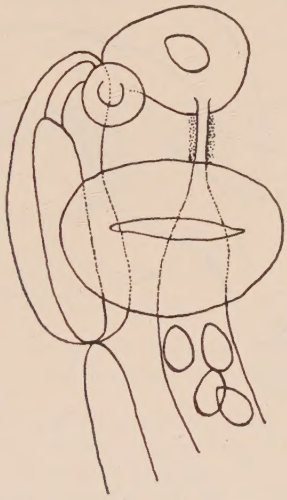

FIG. 7. *Hairana sohali* n. g., n. sp. Terminal end of genital ducts.

Hairana magnus n. sp.
(Figs. 8 & 9)

Four specimens were obtained from *Acanthurus sohal*. It is a larger species than the previous one, being about twice its length. The specimens range from 4.25 to 5.5 mm in length and 1.032 to 1.204 mm in maximum breadth. The cuticle is beset with spines which measure 0.014 to 0.018 mm. The ventral sucker is slightly smaller than the oral and measures 0.212 to 0.338 mm in length and 0.189 to 0.383 mm in width. It is situated in the posterior part of the anterior quarter of the body length, being in a more anterior position than in the previous species. It is lateral to the median line, to the right in three specimens and to the left in one. The ventral sucker is provided with six to eight rows of scales which are lacking in the previous species.

The digestive system: The oral sucker is situated ventrally at the extreme anterior end of the body and measures 0.315 to 0.405 mm antero-posteriorly and 0.293 to 0.338 mm transversally. There is a short pre-pharynx and a well-developed muscular pharynx measuring 0.203 to 0.284 mm and 0.194 to 0.248 mm in length and width, respectively. It is followed by a fairly long and wide oesophagus which bifurcates at about the level of the ventral sucker into two simple intestinal caeca which reach the posterior end of the body. No union and no anus could be detected.

The male genitalia include a single testis which occupies the anterior half of the posterior third of the body intercaecally. It is elongated antero-posteriorly, smooth contoured, and 0.616 to 0.968 mm long and 0.298 to 0.396 mm wide. The cirrus sac occupies a median position at about the level of the ventral sucker.

The female genitalia include a spheroid, smooth ovary which is anterior to the testis and intercaecal in position. It occupies the posterior part of the fifth 8th of the body length and is 0.225 to 0.428 mm in diameter. There is no receptaculum seminis. The uterus occupies a wider area than in the preceding species, is mainly inter-caecal and ends in a metraterm pouch which

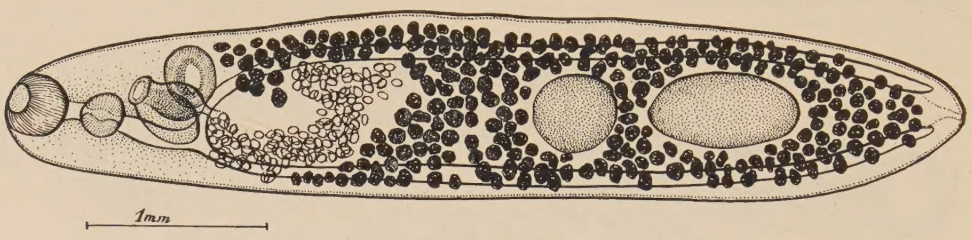

FIG. 8. *Hairana magnus* n. sp. Ventral view.

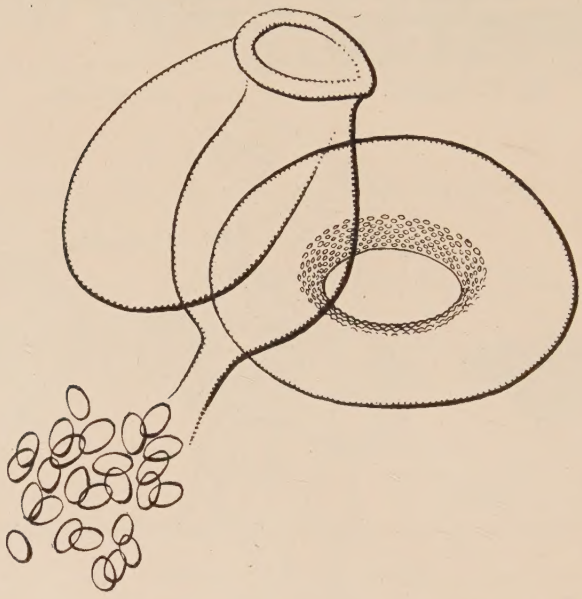

FIG. 9. *Hairana magnus* n. sp. Ventral sucker and terminal end of genital ducts.

opens into a common genital atrium anterior to the ventral sucker, slightly to one side of the median plane. Ova are ovoid and measure 0.054 to 0.063 by 0.05 to 0.054 mm in diameter.

The excretory system, as in the previous species, could not be made out with the exception of its terminal opening posteriorly.

This species differs mainly from the previous one in being larger, the ventral sucker is situated in a more anterior position, lateral to the median line and is provided with circular rows of scales.

The genus *Hairana* is characterized by having an oral sucker without processes; pre-pharynx, pharynx and oesophagus are present, intestinal caeca long and apparently do not unite posteriorly; single acetabulum, in the anterior third of body, is of about the same diameter as oral sucker; single testis elongated antero-posteriorly, occupying anterior part of posterior third of body, cirrus sac well developed, vesicula seminalis interna and externa present; ovary entire and spheroid, no receptaculum seminis could be detected; vitellaria extensive, distributed throughout the body posterior to acetabulum, uterus entirely pre-ovarian and contains many ova; it opens into a metraterm pouch.

Type species: *H. sohali*

Other species: *H. magnus*

Discussion.—In 1910 Linton created the genus *Enenterum* to include *E. aureum* from *Kyphosus sectatrix,* and in 1942 the author described a second species, *E. pimelopteri* from *Pimelopterus tahmel.* In 1946 Dollfus described *E. pseudaureum* from the same host as Linton's; this species is identical with the species described by the author and is considered a synonym of it. Dollus, in a private communication, stated that he was not aware of the author's publication, and Manter (1947) is of opinion that *E. pseudaureum* is probably a synonym of *E. pimelopteri.* In the same paper Dollfus described two new sub-genera of *Enenterum,* namely *E.* (*Cadenatella*) *cadenati* and *E.* (*Jeancadenatia*) *brumpti.* Manter (1947) rightly suggests raising these sub-genera to generic rank.

The two genera described above by the author, i.e., *Spiritestis* and *Hairana* seem to be allied to *Enenterum, Cadenatella* and *Jeancadenatia,* from which they can be differentiated by the following key.

Oral sucker provided with lobes:
- No accessory suckers present:
  - With 10 pre-oral lobes, long intestinal caeca, with an anal opening posteriorly; two testes at posterior half of body, vesicula seminalis interna only present ..... *Enenterum*
  - With 4 pre-oral lobes, short intestinal caeca that end blindly posteriorly; one large testis that occupies posterior half of body, vesicula seminalis interna and externa present ..... *Spiritestis*
- Accessory suckers present:
  - With 8 pre-oral lobes, intestinal caeca long; one accessory sucker anterior to acetabulum; one testis at middle of body ..... *Cadenatella*
  - With 10 pre-oral lobes, intestinal caeca apparently long; 14 or 15 accessory suckers anterior to acetabulum; two testes occupying 3rd quarter of body ..... *Jeancadenatia*

Oral sucker not provided with lobes:
- Intestinal caeca long; one testis occupying anterior part of posterior third of body; vesicula seminalis interna and externa present..... *Hairana*

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STUDIES ON SOME PARASITIC MITES FROM CANADA AND SOUTH AFRICA

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INTRODUCTION

In August 1947, Mr. V. R. Kaschula of the Allerton Government Laboratory, Pietermaritzburg, sent me some mites for identification which had been taken from the tracheae of cage canaries in Pietermaritzburg; these have been described as a new species, since they do not agree with any of the rather brief, and in many cases unillustrated accounts of the few species of Rhinonyssidae that have been described.

The second new species in this paper is a parasite of the North American beaver, and belongs to the family of fur-mites or Listrophoridae. I am greatly indebted to Mr. H. H. J. Nesbitt of the Division of Entomology Department of Agriculture, Ottawa, who was the means of enabling me to see these specimens and who went to considerable trouble in order to obtain them.

I have taken this opportunity of reviewing the subfamily Labidocarpinae, recently proposed by Gunther, and enlarging it; the task has been greatly lightened by the kindness of Dr. Marc André, of the Museum National d'Histoire Naturelle of Paris, who not only sent me the type slides of Trouessart's three species of *Ladidocarpus* but also descriptions from literature which is unobtainable in South African libraries.

Two new records of Sarcoptoid parasites occurring in South Africa have been included in the paper.

PARASITOIDEA

Family Rhinonyssidae Trouessart

Sternostoma Berlese and Trouessart, 1889.

Sternostoma Berlese and Trouessart, 1889. Bull. Biblioth. Sci. de l'Ouest, Annee 2: 126.

A number of mites taken from the trachea of canaries by Mr. V. R. Kaschula, who found them firmly embedded in the inner walls of the organ and covered with mucus, appear to belong to this genus. The host birds had been sent in to the Allerton Laboratory by their owner for investigation, since a number of them were losing condition and emitting distinct coughing or wheezing noises from time to time. One or two of the canaries died during a cold winter spell in July, 1947, though the mortality in these cases was more probably due to the indirect effect of the mites on the general health and powers of resistance of the birds. The short length of trachea when dissected revealed a large number of mites (30–40) attached to its inner walls and the intestines of these parasites were clearly filled with blood. It was found very difficult to clear away the mucus in which the mites were embedded and to make satisfactory mounts. When treated too long with clearing agents, the mounted specimens tended to lose their shape and it was thus difficult to ascertain the correct relative sizes of the legs; if not cleared long enough the mucus was not sufficiently removed, rendering the microscopic structure of the specimens obscure.

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Judging from the literature available, the parasites of this family all seem to have been taken from the nasal passages or cavities of birds. The mites described below however were definitely attached and feeding inside the trachea throughout its entire length, as far as the origin of the bronchi; none were found in the nasal cavities, though it is possible that a number were present but were overlooked. It is even possible that the mites in the tracheae were stragglers from the main body, and after being crowded out of the nasal areas, descended the trachea.

The diseases of canaries are discussed in a chapter of "Canaries and Cage Birds"

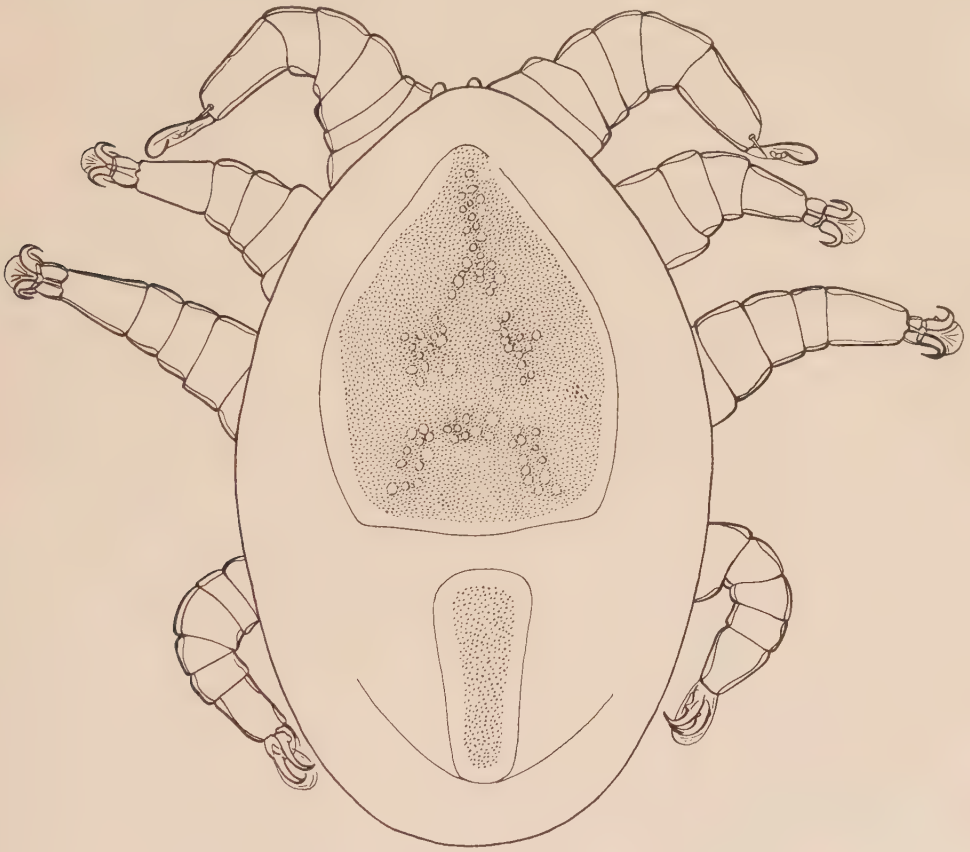

FIG. 1. *Sternostoma tracheacolum* n. sp. ♀. Dorsal surface.

(Blakiston, Swaysland and Wiener), by Dr. W. Gordon Stables, but the causes of respiratory diseases are considered by this author to be natural ones, such as exposure to damp and cold, and no mention is made of mite parasites or other causative organisms.

No mites from the tracheae or nasal cavities of canaries seem to have been previously recorded and the species in question probably has its closest relationships with *Sternostoma cryptorhynchum,* a mite described by Berlese and Trouessart (1899) from the nasal cavities of the European sparrow, *Passer domesticus.* It certainly seems to belong to the genus *Sternostoma,* which according to Vitzthum's (1935) key, is distinguished by 2 dorsal shields, the mouthparts invisible from above, and the absence of peritremes in the stigmata.

Types: 23 specimens mounted in Berlese's modified medium.

Colour before clearing, yellow-brown.

Seen from above, Fig. 1, the mouthparts are either invisible or only their extreme apices can be seen.

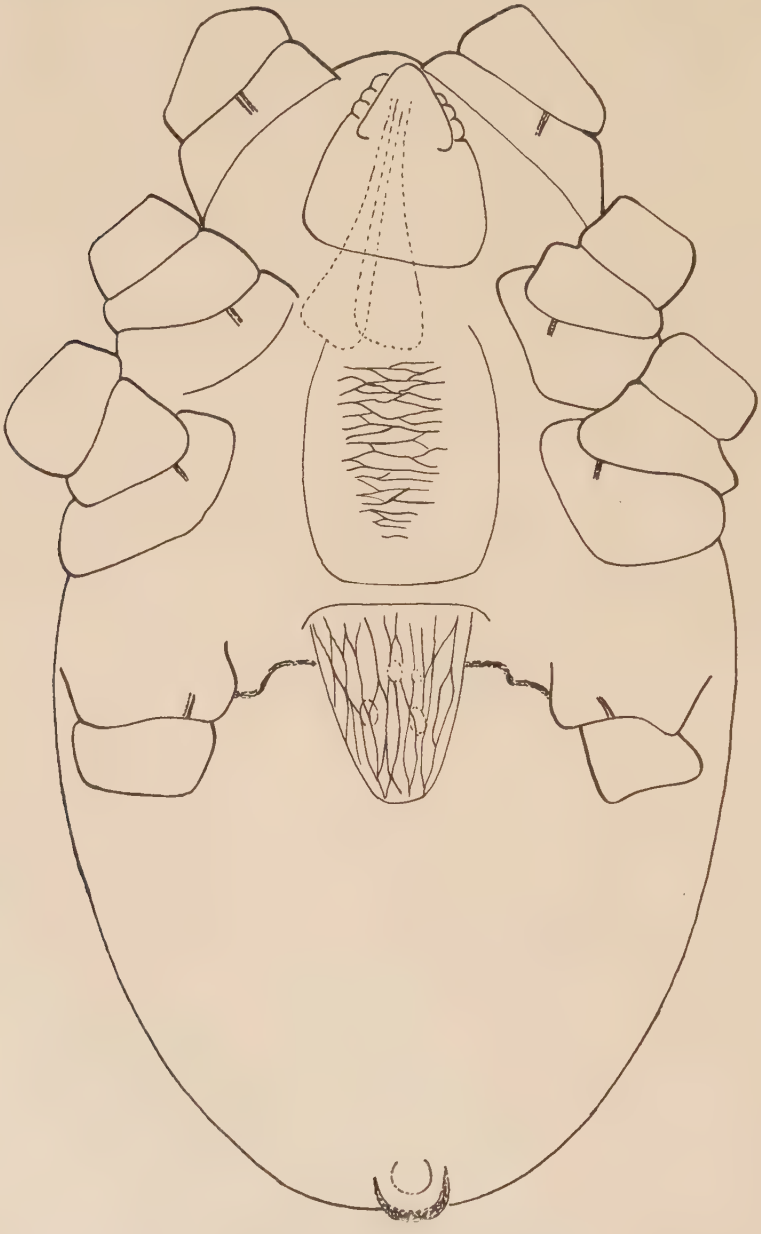

FIG. 2. *Sternostoma tracheacolum* n. sp. ♀. Ventral surface.

Sternostoma tracheacolum n. sp.
Figs. 1–3

Dorsal surface with two shields, the anterior one large and pentagonal, more or less pointed anteriorly; its entire area appearing finely stippled or granular under magnification, with a number of small clear alveoli forming a definite symmetrical pattern; posterior shield rod-shaped, rather wider anteriorly than posteriorly, uniformly finely granular; no anal shield.

Ventral surface as in Fig. 2, the shields very ill-defined, the sternal plate with transverse, the genito-ventral plate with longitudinal striations.

Body and legs perfectly smooth, without spines, except for one small spine near the base of the claw on dorsal surface of leg I (Fig. 3b). This spine absent from the tarsi of legs II–IV. Legs very strong and thickset, varying in length owing to the stretching and telescoping of these structures in mounted specimens, but leg I usually distinctly longer than the others.

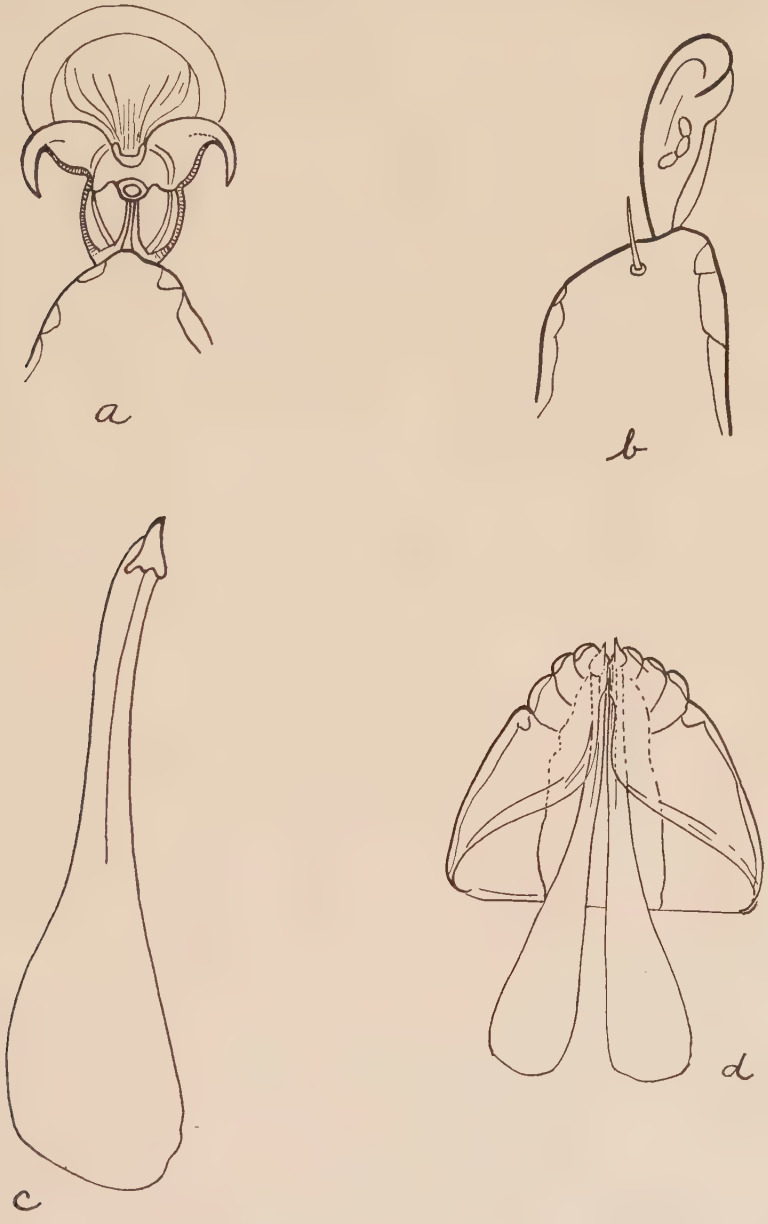

FIG. 3. *Sternostoma tracheacolum* n. sp. ♀. *a*, claw of leg III; *b*, claw of leg I; *c*, chelicera; *d*, mouthparts seen through the transparent skin of dorsal surface.

Claws of leg I (Fig. 3b) much modified and quite different from those of the remaining legs in which the 2 main branches of the claw are very strong and distinct (Fig. 3a). In the first leg these have largely disappeared, the one ramus being apparently represented by the

thickened boundary of the dorsal surface which is also much curved distally, the other ramus only represented by indistinct traces. Judging from the figures of various species of RHINONYSSIDAE given by Vitzthum (1935) some of them have the claw of leg I modified, in others the claws of all four legs are similar. This modification seems to have gone even further in the species under consideration than in any of those illustrated by Vitzthum.

Mouthparts. The chelicerae (Figs. 3c and 3d) are large and seem to be very mobile as in some mounts they occupy positions far forward, in others they are much retracted. The apices appear to have a barbed structure which doubtless enables the mite to retain its hold when the chelicerae are embedded.

Seen from above through the transparent dorsal integument the chelicerae and palps are enclosed in a kind of hood, the two halves of which bend upwards and over, meeting or nearly touching in the middle line. Palpi strong and thickset, the apical segment strongly rounded, all the segments with lateral chitinization.

Dimensions: Length 490 μ, width 300 μ.

It might be possible to consider the above described form as identical with the solitary species of this genus, *S. cryptorhynchum* Berlese and Trouessart (1889, p. 127). The general shape of the dorsal shields seem to agree with Berlese and Trouessart's account, which is however rather brief, lacks detail and includes no illustrations. The authors moreover expressly state that in their species the claws of all four legs are similar, differing in this respect from the genus *Leiostaspis.* Further differences in the species under consideration are the fairly robust palpi (those of *S. cryptorhynchum* are described as minute), and the generally larger size of the mite.

All our specimens appear to be females, judging by the structure of the ventral shields. It is remarkable that the majority of Rhinonyssid species have been based on female specimens, the males being unknown; this is clearly seen in Vitzthum's paper, previously quoted.

SARCOPTOIDEA

In 1944, while describing a new form of mite, *Schizocoptes conjugatus,* from the South African Golden mole, I wished to compare it with a specimen of *Schizocarpus mingaudi* from the beaver, which, from the key given by Canestrini and Kramer (1899, p. 24), seemed to be the form most closely allied to it. One of the few illustrations of this species appears in N. Banks' report on the Acarina (1915, p. 127, Fig. 261) and on the previous page is another illustration (Fig. 260) bearing the caption *Schizocarpus mingaudi,* also described as occurring on the beaver. This last, however, obviously cannot be the same mite as that shown in Fig. 261, and belongs to a quite different genus. The mite shown in Banks' Fig. 260 is certainly a species of *Labidocarpus,* or a genus belonging to the same group; in order to throw some light on the matter I asked Mr. H. H. J. Nesbitt of the Division of Entomology, in the Department of Agriculture, Ottawa, to send me some fur-mites from the Canadian beaver. This he very kindly did, sending me an excellent slide with 4 mounted mites from the muzzle hairs of *Castor canadensis.* These mites are obviously the same as those portrayed by Banks in his Fig. 260 and belong, if not to the genus *Labidocarpus,* which they resemble in the general form of the body, to a genus closely allied to it.

I have decided to place this species of Listrophorid mite in a new genus, for reasons which will appear in the following description and in the key to the subfamily LABIDOCARPINAE below.

Family LISTROPHORIDAE

Prolabidocarpus n. gen.

A description of the generic characters is given below in the section dealing with the revision of the subfamily LABIDOCARPINAE Gunther.

Prolabidocarpus canadensis n. sp.
Figs. 4, 5.

Types: 4 specimens, evidently all females, one of them containing a hexapod embryo in the body cavity (Fig. 4).

Head shield of the usual *Labidocarpus* form, with strong and complex chitinization. Anterior legs also strongly chitinized, I rather larger than II, both legs at their apices consisting of a large outer and small inner plate between which the hair passes, thus serving as an adhesive device.

Posterior legs as in Fig. 5b, 5c, the tarsi of both without claws, but with circular sucking discs, the penultimate segment of leg III (Fig. 5b) with a stout spine, absent from the same segment in leg IV. These legs strongly built, shorter than in *Alabidocarpus,* and well chitinized.

FIG. 4. *Prolabidocarpus canadensis* n. sp. ♀. The transverse annulations of the skin not shown.

Above the insertion of leg III a fairly short seta and more anteriorly, as seen in Figs. 4, 5d, two others a short distance behind the posterior margin of the head shield. These setae about one fifth the length of the long setae at the posterior apex of the body. Below the long posterior abdominal setae another pair which are about twice as long as the first mentioned anterior lateral setae, and below them again a pair of minute setae.

Body with 60–63 annulations.

Body cavity of one ♀ (Fig. 4) containing a hexapod embryo with its head directed posteriorly; this embryo resembling the adult in miniature except that the fourth pair of legs has not yet appeared.

Length of largest ♀ (containing hexapod embryo), 460 μ, depth 190 μ.

A REVISION OF THE SUB-FAMILY LABIDOCARPINAE GUNTHER

Several attempts have been made to enlarge the original genus of Trouessart, *Labidocarpus,* by breaking it up and by the addition of other genera, the latest being that of Gunther who, in a revision of the family Listrophoridae (1942) has divided it into 4 sub-families; one of these, the LABIDOCARPINAE, contains two genera of the *Labidocarpus* group and two other non-allied genera, *Chirodiscus* and *Schizocarpus.*

The various forms which have been described under the name of *Labidocarpus* have in their general appearance, laterally compressed bodies, shovel-like head shields, and the two highly modified anterior legs, much in common, and their

general habitus would incline the taxonomist to regard them as all belonging to one genus. All of them at any rate form a group of species quite distinct from such genera as *Schizocarpus* and *Chirodiscus,* also included in the LABIDOCARPINE subfamily.

This general similarity however is no longer so obvious when the various species are examined in more detail, especially with regard to the end-structures of the tarsi of legs III and IV, and also with respect to a number of other characters, such as the number of body rings and arrangement of the setae.

FIG. 5. *Prolabidocarpus canadensis* n. sp. *a,* leg I enlarged; *b,* leg III; *c,* leg IV; *d,* anterior region of body in a different specimen from that shown in Fig. 4.

Ewing was fully justified (1929) in separating the genus *Alabidocarpus* from Trouessart's original *Labidocarpus,* which should be based on the species *L. rollinati.* Ewing's proposal was to include Trouessart's two other species, *L. megalonyx* and *L. minor,* together with a species of his own, *L. compressus,* in this genus, since in all of them the third pair of legs ended in "one long and two short claw-like structures." The tarsus of legs III and IV in *L. rollinati* has quite different terminal structures, as can be seen by comparing leg III in *L. rollinati* (*Labidocarpus*) with that of a representative of *Alabidocarpus* (*L. nasicolus* Lawr.), Figs. 6a and 6b. In *L. rollinati* the tarsus ends in a fairly pointed claw at its extreme apex; on its under

side, and projecting almost at right angles to the main claw, is a smaller subsidiary claw or claw-like process, fairly short and pointed. It is thus unjustifiable for Gunther to doubt the necessity for Ewing's new genus, *Alabidocarpus,* created "on the strength of very minor differences in the spurs on leg III" (Gunther, 1942, p. 110. If Figs. 6a and 6b are compared it will be seen that these are by no means minor differences; the tarsi of *Labidocarpus rollinati* on the one hand, and those of the genus *Alabidocarpus* proposed by Ewing, which should include the species *A. megalonyx, minor, nasicolus* and *recurvus,* are fundamentally different in their structure. This is already apparent in Trouessart's original descriptions and illustrations of *L. rollinati* and *L. megalonyx.* Gunther's statement is supported by Womersley (1943, p. 17) who also says that Gunther's *Neolabidocarpus* and the group of species coming under Ewing's *Alabidocarpus* have 5 segments in legs III and IV. He then makes the following surprising statement "In this they differ from the European species which as figured by earlier workers have only 3-segmented legs III and IV." Any mite with only 3 segments to a leg would be a curious abnormality but especially so in the LABIDOCARPINAE where the unmodified posterior legs have largely retained their normal structure. The European species were described by Trouessart in Bull. Soc. Ent. France, 1895, pp. 83–87, and this accurate and extremely observant worker not only figures his *L. rollinati* with 4 segments, but expressly states that the number is 4 in his description of the genus, "pattes de la 3^{e} paires normales, de 4 articles" (p. 84).

I have, owing to the courtesy of Dr. M. André of the Museum National d'Histoire Naturelle of Paris, been given the opportunity of examining Trouessart's type slides and can confirm the accuracy and care with which this author made his drawings and descriptions. In one respect only I should be inclined to disagree with Trouessart's conclusions, and that is relating to the number of segments in the posterior legs of *L. megalonyx* and *L. minor.* The number may be open to a difference of interpretation, but in these species, as well as in *L. nasicolus* and *L. recurvus* as stated by Womersley (loc. cit.), the posterior legs do seem to differ from those of *L. rollinati* in having 5 rather than 4 segments.

Gunther was probably misled by Ewing's (1929) drawing of his *Labidocarpus (Alabidocarpus) compressus,* which is shown (Fig. 27) as having only 3 segments in legs III and IV, but if he did accept this as representing the correct number, it was illogical of him to place *Labidocarpus nasicolus, L. megalonyx* and *L. minor* in the same genus, since these species have more than 3 segments.

Seeing that *L. rollinati* has only 4 segments in legs III and IV (Fig. 6a), and *L. megalonyx* and *L. minor* 5, this is a further reason for separating the two latter species from the parent genus *Labidocarpus,* which should contain only the one species, *L. rollinati.* These two species should therefore be placed in the genus *Alabidocarpus* Ewing, together with *L. nasicolus* Lawrence and *L. recurvus* Womersley; all these have 5 segments in legs III and IV and the same type of tarsal end-structures, i.e. a single curved claw and 2 conspicuous thickened spurs in III, a slender and very long curved claw and 1 spur in leg IV.

Ewing suggests that his *Labidocarpus compressus* should also be included in the genus *Alabidocarpus* on the same grounds, i.e., the tarsal structure of legs III and IV, but whether it is possible to do so appears doubtful from the following considerations of his species.

The original description of the type (1910) is brief and the only illustration is an extremely poor microphotograph, which, while it conveys an idea of the general shape of the mite, and the relative position and proportions of the legs and main setae, gives no detailed information of the various structures. The figure given in

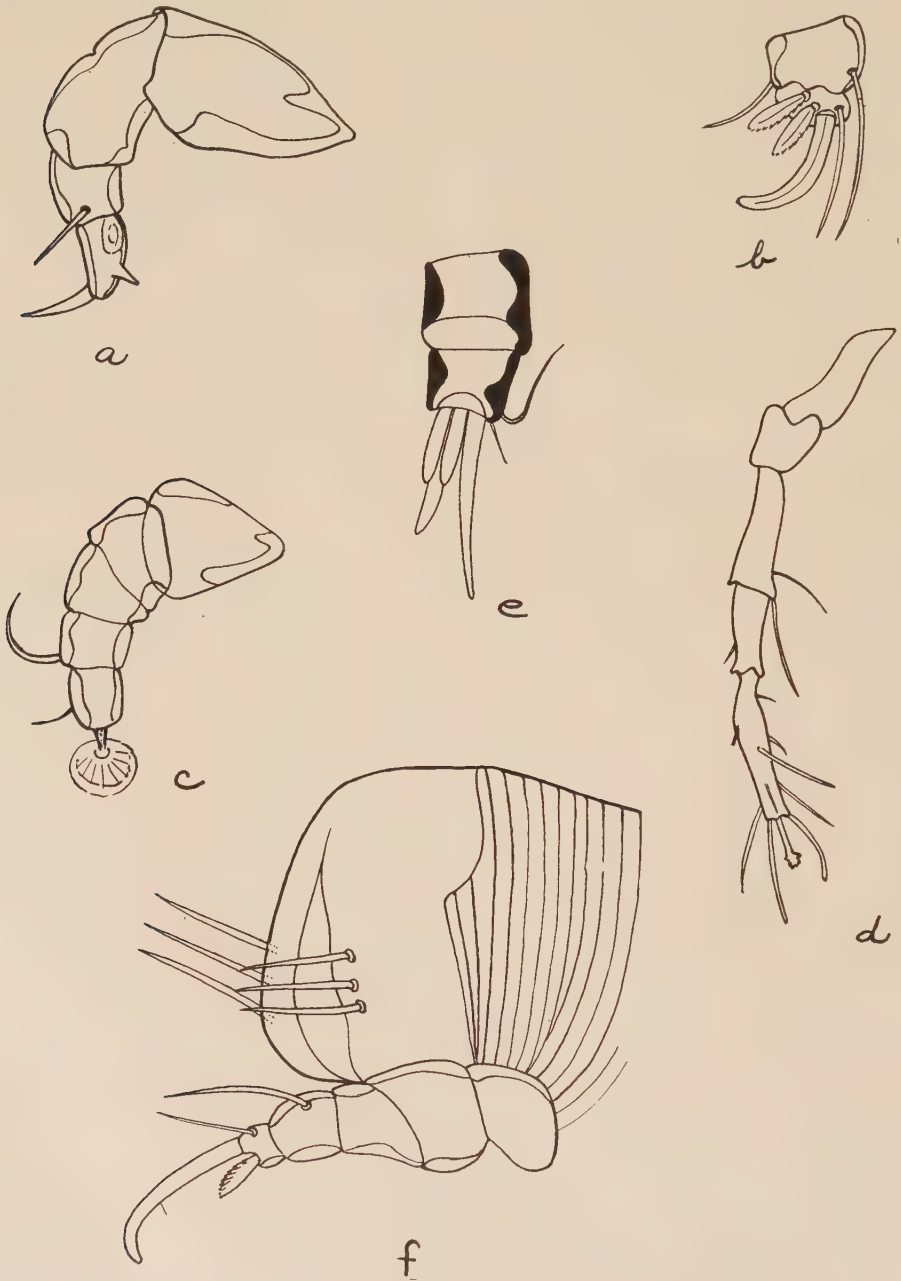

FIG. 6. *a*, leg III of *Labidocarpus* (*L. rollinati*) ; *b*, leg III of *Alabidocarpus* (*A. nasicolus*) ; *c*, leg III of *Prolabidocarpus* (*P. canadensis*) ; *d*, leg IV of *Neolabidocarpus* (*N. buloloensis*) after Gunther ; *e*, leg III of *Olabidocarpus* (*O. belsorum*) after van Eyndhoven ; *f*, posterior apex of abdomen of ♂ of *Alabidocarpus megalonyx* (Trouess.).

"The Manual of External Parasites" (1929) is rather better than the original illustration, but is still extremely generalized.

There are several curious features about the species as illustrated by Ewing which makes it certain that either (a) this form cannot be placed in the genus *Alabidocarpus* as suggested by Ewing, or (b) his illustration is incorrect.

The first abnormality in Ewing's second figure in the 1929 description is that the third and fourth legs have only 3 segments, a state of affairs which is virtually impossible in a mite of this sub-family; a less striking feature about these legs is their extremely small size, being actually shorter than the modified anterior ones. Although these legs are never particularly large in the genus *Alabidocarpus,* they are always at least a little longer than the paddle-like anterior legs, generally considerably longer; their remote position from these anterior legs, i.e., forming a separate group near the posterior end of the body, is also not found in *L. megalonyx, minor, nasicolus* or *recurvus.*

The appearance of the anterior legs with their extremely simplified structure, is also unusual. These legs in Labidocarpine genera are rather complex, with an ill-defined but strongly chitinous structure which is very difficult to reproduce in an illustration. It is by no means easy to define the joints between the greatly modified segments of these legs, though they can be indistinctly seen in some specimens when they are revealed by some chance angle of mounting. It is difficult to believe that in this species these anterior legs are merely simple paddle-shaped outgrowths of the body, as would appear from Ewing's figure.

Another difference which marks off this species from members of the genus *Alabidocarpus* is the position of the large lateral spine which is shown a short distance posterior to leg II. In the remaining species of *Alabidocarpus* this spine is found just above the insertion of the *third* leg.

Other features which distinguish it from all other Labidocarpine genera are the curious neck-like constriction of the body behind the anterior legs, and secondly the two pairs of very long and strong spines which spring from the posterior margin of the anterior chitinous head scute.

A kind of suture which in Ewing's later illustration runs backwards from the second leg along the side of the body, and separates off what appears to be a large ventral scute from the rest of the body, would be quite unnatural if found in any other species of *Alabidocarpus.*

The apical structures of the tarsi in legs III and IV, which can just be seen in this illustration, constitute the only certain character which this species has in common with the other species of the genus. It is, however, an extremely important one, and in this respect *compressus* certainly does agree more closely with the species which Ewing has assigned to the genus *Alabidocarpus,* than to any other genus. Notwithstanding this agreement however, it seems to be the writer impossible to include Ewing's *compressus* in the same genus as *megalonyx* and the other species for which he proposed the name *Alabidocarpus.* A new genus, *Eulabidocarpus,* is therefore proposed to receive *Labidocarpus compressus,* on the score of the many differences of structure which it reveals, apart from considerations of the tarsi of legs III and IV.

It is proposed to extend the *Labidocarpus* group of pilicolous mites by following Ewing in dividing the original genus *Labidocarpus* into two, and adding further

genera; all of these may perhaps be more conveniently regarded as subgenera, since they have a number of characters in common which distinguish them from the remaining genera of the subfamily LABIDOCARPINAE, such as *Chirodiscus, Schizocoptes* and *Schizocarpus.*

These general characters are (1) the lateral compression of the body (2) the highly modified and shortened anterior legs which are without caruncles; these legs are paddle- or flap-like and are dilated distally into a larger outer and smaller inner flattened plate which are applied to the hair to which the mite clings, (3) the numerous vermiform annulations and almost transparent skin of the body (4) the large epistome which at the anterior end of the body, forms an almost solid chitinous plate, more or less triangular in shape.

The females are viviparous, often containing a single hexapod embryo in the body cavity.

The characters on which it is proposed to base the various genera are mainly the differences of structure in the terminations of tarsi III and IV, though a number of other characters are also of importance. The following salient features should be sufficient to distinguish the various genera.

1. *Labidocarpus* Trouessart, 1895

Genotype: *Labidocarpus rollinati* Trouessart.

L. rollinati Trouessart, 1895, Bull. Soc. Ent. France, 85, Figs. 1, 2.

Legs III and IV fairly short and thickset, with 4 segments, the tarsi of both legs similarly armed with a moderately long claw at the extreme apex, a second pointed subapical claw or process on the ventral surface, directed at right angles to the larger apical claw. Leg III distinctly longer than IV. The number of annulations of the body small, about 30 in both sexes. The sexes strongly heteromorphic, the male with a marked excavation at the posterior apex of the venter which is covered with a subdivided chitinous plate bearing a pair of adhesive discs. Length of ♂, 270 μ; of ♀, 350 μ.

The genus contains a single species *L. rollinati* Trouessart.

2. *Alabidocarpus* Ewing, 1929

Genotype: *Labidocarpus megalonyx* Trouessart.

L. megalonyx Trouessart, 1895, Bull. Soc. Ent. France, 87, Fig. 3.

Legs III and IV fairly short and thickset (though considerably longer than the modified anterior legs), with 5 segments. Tarsi of legs III and IV quite differently formed from those of Labidocarpus and III armed differently to IV. Tarsus III ending in a moderately long curved claw (longer than the tarsal segment) with 2 much shorter thickset spurs at its base; tarsus IV ending in a similar but much longer curved claw (much longer than the length of the tarsal segment) with a single spur at its base. Legs III and IV subequal. The number of annulations of the body usually large, 40–90 in the ♀, 20–40 in the ♂. A large lateral seta just above the insertion of leg III, only a little shorter than the pair of long setae at the posterior apex of abdomen.

The sexes not strongly heteromorphic, ♂ without adhesive discs. Females often containing a single hexapod larva in the body cavity, of much the same general structure as the adult, the fourth pair of legs absent.

The genus at the present time contains 4 species which can be distinguished by means of the following key:

1. Body of ♀ with 40 annulations; size small, 280 μ (♂), 300 μ (♀); body of ♀ long and slender ..... *minor*
   Body of ♀ with 60 or more annulations, size larger, 600–900 μ (♀); body of ♀ more thickset 2
2. Body of ♀ with about 90 annulations, tarsi III and IV longer than wide ..... *recurvus*
   Body of ♀ with about 60 annulations, tarsi III and IV wider than long or as wide as long .. 3
3. Length of ♀ 900 μ, spurs of tarsus III about 1/3 length of claw, claw of tarsus IV much curved apically ..... *megalonyx*

Length of ♀ 640 μ, spurs of tarsus III about 1/2 length of claw, claw of tarsus IV less curved apically ..... *nasicolus*

The ♂ of *minor* resembles that of *megalonyx* in general, notably in the structure of the posterior margin of the abdomen; this in both species consists of a broad, strongly chitinized band (Fig. 6f); *megalonyx* however is $1\frac{3}{4}$ times as long as *minor,* and the number of annulations twice as many, 40 as compared with 20. Figure 6f represents the posterior part of the abdomen in *megalonyx;* in *minor* there appears to be only a single pair of spines in the lower half of the posterior margin of the abdomen, these much longer than the 3 pairs seen in *megalonyx.* In Trouessart's type slides of the two species which I have examined, the minute structures are not altogether clearly defined; they appear to have become clogged with the mounting preparation, which has deteriorated since the mounts were made more than 50 years ago.

3. *Olabidocarpus* n. gen.

Genotype: *Labidocarpus belsorum* van Eyndhoven.

L. belsorum van Eyndhoven, 1940, Zool. Anzeiger, 130, H. 9/10: 230.

Legs III and IV with 5 segments, the tarsi of the same fundamental structure as in *Alabidocarpus,* i.e., tarsi III and IV have a long curved slender claw with shorter spur-like structures at its base.

They differ in having 3 spurs on tarsus III (Fig. 6e), 2 on tarsus IV, instead of 2 and 1, respectively (*Alabidocarpus*); the long curved main claw subequal in the two legs (in *Alabidocarpus* that of IV considerably longer than III). Leg III distinctly larger than IV.

The structure of the head shield quite different from that of *Alabidocarpus,* the posterior end being divided somewhat as in *Labidocarpus rollinati,* with a backward projection from each postero-lateral angle. In the median line 2 long narrow shields passing backwards to a little beyond the level of leg III; anteriorly to these shields and between them and the main scute, a pair of long setae; the lateral projections of the head shield with a much shorter seta at their apices. The chaetotaxy of the head shield perhaps resembles that of *Eulabidocarpus compressus* in which there are also 2 pairs of setae, the one strong the other moderate.

The lateral seta above the insertion of leg III only moderate. Body of ♀ with only 40 annulations; length, 365 μ. Only the ♀ of this species is known.

I find Van Eyndhoven's key to the various species of *Labidocarpus* (sens. lat.) rather obscure and difficult to follow (loc. cit. p. 233). He is incorrect in saying that there is no lateral seta above the insertion of leg III in the ♀ of *megalonyx* (line 13). This is present in the types of both sexes which I have examined and is little shorter than the large setae at the posterior apex of the abdomen.

4. *Eulabidocarpus* n. gen.

Genotype: *Labidocarpus compressus* Ewing.

L. compressus Ewing, 1910, Trans. Acad. Sci. St. Louis, **19:** 120, Pl. 35, Fig. 4.

Legs III and IV with 5 (?) segments (3 according to Ewing), subequal in length and very short, shorter than the modified anterior legs and forming a separate group far removed from them near the posterior end of the body.

Tarsi III and IV with the same end-structure as *Alabidocarpus.*

Body with about 50 (?) annulations, a distinct constriction behind the level of the second pair of anterior legs. Head scute with 2 pairs of setae on its posterior margin, one of them very strong and sub equal in length to the pair at the postero-dorsal end of the abdomen.

One large lateral seta behind leg II and much nearer to it than to leg III, subequal to the postero-dorsal setae. Length 440 μ. The genus contains a single species, *E. compressus* (Ewing).

5. *Neolabidocarpus* Gunther, 1942

Genotype: *Labidocarpus buloloensis* Gunther.

L. buloloensis Gunther, 1940, Proc. Linn. Soc. New So. Wales **65:** 353, Figs. 1–5.

The genus differs completely from all others of the *Labidocarpus* complex in the structure of legs III and IV, which are much longer and more slender than in any other form. The seg-

ments of these legs much longer than wide and provided with a number of long and slender setae (Fig. 6d). Legs III and IV consisting of 5 segments, the tarsi ending in a single claw-like structure somewhat swollen at its apex. Body with numerous annulations (160–170 according to Gunther's figure). The sexes heteromorphic, the male with 26 setae, all short, the female with 8, some of them long. Length of ♀, 292–325 μ; of ♂, 375–542 μ.

The genus contains a single species, *N. buloloensis* (Gunther).

Gunther states that the tarsus is provided with 2 slender apical claws and a shorter empodium between them. I should be inclined to doubt the existence of 2 claws; the structures designated by Gunther as such appear in his figure to be very similar to the remaining setae of the leg and I should interpret them as such, while his empodium should be regarded as the claw. It is extremely unlikely that a member of this family would have two claws on the posterior legs.

6. *Prolabidocarpus* n. gen.

Genotype: *Prolabidocarpus canadensis* Lawrence.

Resembling the *Labidocarpus* form in the strong lateral compression of the body, the modified paddle-like anterior legs, and the general appearance of the body.

The genus differs from all others in the complete absence of claws on the tarsi of legs III and IV (Figs. 5b, 5c) and the presence of distinct and well defined circular sucking discs (*haftlappen, ventouses ambulacraires*). Legs III and IV thickset and rather short as compared with those of *Alabidocarpus* Ewing, with 5 distinct segments. In its body form this genus resembles *Alabidocarpus* but the long lateral seta above the origin of leg III is replaced by a much shorter one; more anteriorly behind the posterior margin of the head shield there are two others of approximately the same size (Fig. 4). Body with about 60 annulations in the ♀, the ♂ of this genus not known. Reproduction viviparous, as in *Alabidocarpus,* a single hexapod embryo being contained in the body cavity of the ♀. Size of ♀, length 460 μ, depth 190 μ. The genus contains a single species, *Prolabidocarpus canadensis* Lawrence.

Subfamily Labidocarpinae Gunther
Type of subfamily, *Labidocarpus* Trouessart, 1895

Legs I and II modified as claspers in conformity with their function of grasping the hair, without caruncles. Legs III and IV with or without caruncles; though not modified they are in some cases used for holding the hair.

The subfamily contains 9 genera including *Schizocoptes,* a genus recently described by Lawrence (1944) from the fur of the South African Golden mole, *Bematiscus (Chrysochloris) villosus dobsoni* (Broom).

Key to the Genera of Labidocarpinae

1. Body more or less flattened in a dorso-ventral direction 2
   Body laterally compressed 4
2. Third leg much larger than fourth ..... *Schizocarpus* (1)
   Third and fourth legs subequal 3
3. Two anal suckers, leg I enlarged apically, the terminal segment cleft ..... *Schizocoptes* (2)
   No anal suckers, leg I fairly long, subparallel, curved and hollowed out on its under side apically ..... *Chirodiscus* (3)
4. Legs III and IV with 4 segments ..... *Labidocarpus* (4)
   Legs III and IV with 5 segments 5
5. Tarsi of legs III and IV with ambulacral suckers but no claw (Fig. 6c) . *Prolabidocarpus* (5)
   Tarsi of legs III and IV without ambulacral suckers, a distinct claw (Figs. 6b, 6d) 6
6. Segments of legs III and IV fairly short and thickset, the tarsi ending in a long or very long curved claw, and in addition III with 2–3, IV with 1–2 large spur-like structures (Figs. 6b, 6e) 7
   Segments of legs III and IV long and slender, terminal structures of tarsi III and IV quite different (Fig. 6d) ..... *Neolabidocarpus* (6)
7. Posterior legs remote from anterior legs, forming a group near the posterior end of the body, as short as, or shorter than modified anterior legs; the lateral seta close behind leg II ..... *Eulabidocarpus* (7)

Posterior legs not remote from anterior legs, situated as near to the front as to the hind end of the body, or nearer; posterior legs distinctly longer than the modified anterior legs; the lateral seta situated above leg III 8

8. Head shield trifid posteriorly, the middle projection long and narrow; tarsus III with 3, tarsus IV with 2 spurs at the base of the claw (Fig. 6e) ..... *Olabidocarpus* (8)

Head shield not divided posteriorly; tarsus III with 2, tarsus IV with 1 spur at the base of the claw ..... *Alabidocarpus* (9)

THE HOST RELATIONSHIPS OF THE LABIDOCARPINAE

The majority of the *Labidocarpus* group are found on bats, two of them, *Prolabidocarpus* and *Neolabidocarpus,* being parasitic on other animals. *Labidocarpus, Alabidocarpus* and *Olabidocarpus* live on bats of the suborder MICROCHIROPTERA, the two first named genera being confined to various species of the Horse-Shoe bat, *Rhinolophus*, except for one species of *Alabidocarpus, A. recurvus* (Womersley), which without more specific information, is stated to have been found on a bat; it would not be at all surprising if this Australian form were found to occur on *Rhinolophus,* two species of which are known from Australia. *Olabidocarpus* is the parasite of a central European bat, *Myotis myotis,* one of the VESPERTILIONIDAE, a family of bats differing from that to which *Rhinolophus* belongs.

Of the genera that live on CHIROPTERA, *Eulabidocarpus* is the only one known to occur on a bat of the suborder MEGACHIROPTERA. It was taken in Ceylon on the Indian fruit bat but the name of the host, given by Ewing as *Pteropus edwardsii,* should almost certainly be *Pteropus gigantea gigantea.* According to K. Andersen (1), the Indian species of this genus have for years been mistakenly referred to as *edwardsi* (=*rufus*), which is a Malagasy species.

Neolabidocarpus occurs in New Guinea on the scrub wallaby, *Macropus coxeni,* and *Prolabidocarpus* on the Canadian beaver, *Castor canadensis.* The host name of *M. coxeni* given by Gunther may be incorrect as this species of wallaby lives in North Queensland and is not found in New Guinea.

The actual locality of the parasite on the host animal is of great importance since *Alabidocarpus,* except for the Australian species, is known only from the sensory setae of the nose-leaf, whereas *Labidocarpus* is found on hairs from other parts of the body of the Horse-Shoe bat, *Rhinolophus.* In the same way *Prolabidocarpus* attaches itself to the muzzle hairs of the Canadian beaver, *Castor canadensis,* while another quite unrelated Labidocarpine genus, *Schizocarpus,* presumably occurs on the body hairs of the same species of beaver. It therefore seems highly probable that a number of small mammals will be found to support two species of LABIDOCARPINAE, one found in the general fur covering of the host, the other confined to the more specialized hairs, such as the vibrissae of the muzzle region.

Neolabidocarpus is attached to the ordinary hairs of the belly and hind legs of its host, the Wallaby, but in the case of *Eulabidocarpus* and *Olabidocarpus* the region of the body in which the parasite occurs is not known.

Other generalizations which can be made with regard to the habits of the Labidocarpine genera, are whether or not the posterior legs are used for clasping the hair to which the mite attaches itself, in addition to the support afforded by the strongly adhesive anterior legs. In *Alabidocarpus* the posterior legs do hold the hair loosely with the long curved claws of the tarsi which nearly meet or cross each other on the under side of the hair as is illustrated in figure 1b of my paper on

Labidocarpus (Alabidocarpus) nasicolus (1938); this is probably also the case in *Eulabidocarpus compressus* (Ewing).

In *Labidocarpus, Prolabidocarpus* and *Neolabidocarpus,* the posterior legs apparently play no part in fixing the parasite and do not appear to come in contact with the hair. In general this is to be expected since the tarsi of these genera have a less specialized structure; in *Neolabidocarpus* the elongated legs of the female are even folded up and bent upon themselves, so that they are more or less put out of the way.

HOST AND PARASITE LIST OF THE LABIDOCARPINAE

Parasite	*Host*
1. *Labidocarpus rollinati* Trouessart	European Horse-Shoe bat, *Rhinolophus ferro-equinum.*
2. *Alabidocarpus megalonyx* (Trouessart)	"
3. *Alabidocarpus minor* (Trouessart)	"
4. *Alabidocarpus nasicolus* (Lawrence)	Natal Horse-Shoe bat, *Rhinolophus geoffroyi zuluensis.*
5. *Alabidocarpus recurvus* (Womersley)	Australian bat, *Rhinolophus* (?).
6. *Olabidocarpus belsorum* (van Eyndhoven)	European Vespertilionid Bat, *Myotis myotis.*
7. *Eulabidocarpus compressus* (Ewing)	Indian fruit bat, *Pteropus gigantea gigantea*
8. *Prolabidocarpus canadensis* Lawrence	Canadian beaver, *Castor canadensis.*
9. *Neolabidocarpus buloloensis* (Gunther)	Scrub wallaby, *Macropus coxeni,* New Guinea.
10. *Schizocarpus mingaudi* Trouessart	Canadian and European beavers, *Castor canadensis* and *C. fiber.*
11. *Schizocoptes conjugatus* Lawrence	South African Golden mole, *Bematiscus villosus dobsoni.*
12. *Chirodiscus amplexans* Trouessart & Neumann	A bird, *Podargus strigoides,* but more probably an Australian marsupial.

Gunther in his host-parasite list (1942) gives *Chirodiscus* as occurring on the beaver, but as far as I am aware this is incorrect.

NEW RECORDS OF PARASITIC MITES (SARCOPTOIDEA) FROM SOUTH AFRICA

Family ANALGESIDAE

Falculifer rostratus (Buchholz), 1869

A number of hypopial nymphs ("Hypodectes"), from the common pigeon were sent to me by Dr. J. C. van Hille of the Department of Zoology, Rhodes University College, Grahamstown, during September, 1947. They were found in dissected specimens and were described by him as occurring in clusters of as many as 40 on each side of the jugular vein. The specimens correspond exactly to Neumann's figure of the nymph reproduced by Hirst (1922) in "Mites injurious to Domestic animals," Fig. 18 (Economic Series No. 13, British Museum, Natural History).

Family LISTROPHORIDAE

Schizocoptes conjugatus Lawrence, 1944

S. conjugatus Lawrence, 1944, Proc. Zool. Soc. London, 114: 302–306.

A number of specimens from the Golden mole, *Amblysomus (Chrysochloris) hottentotus longiceps* (Broom). The types were recorded from another species of Golden mole, *Bematiscus (Chrysochloris) villosus dobsoni* (Broom).

SUMMARY

1. A new species of Parasitoid mite, *Sternostoma tracheacolum,* is described. The mite was found embedded in the inner walls of the tracheae of cage canaries at

Pietermaritzburg, South Africa, and appears to have an adverse effect on the health of the host.

2. A new pilicolous mite of the family LISTROPHORIDAE (SARCOPTOIDEA) from the Canadian beaver is described; for this species it has been found necessary to propose a new genus, *Prolabidocarpus*. The subfamily LABIDOCARPINAE raised by Gunther in 1942 is revised and enlarged by the addition of two more genera, *Olabidocarpus* and *Eulabidocarpus,* the number for the subfamily being thus increased from six to nine genera. Ewing has been followed in adopting the genus *Alabidocarpus,* proposed by him.

3. Two new records of parasitic mites from South Africa are noted.

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SNAIL HOSTS OF *SCHISTOSOMAJAPONICUM* IN THE SOOCHOW-WUSIH AREA, KIANGSU, CHINA

C. P. Mao[1] and L. Li[2]

The small, amphibious, operculate snail-hosts of *Schistosoma japonicum* have drawn the attention of zoologists as well as epidemiologists as studies on schistosomiasis have been made more extensively in the last three decades. If the bionomics of the gastropod in question is on its way to being elucidated, its nomenclature and taxonomy are still far from satisfactory, if not more confusing than before. In reviewing the literature dealing with the description of these snails, one finds that the denticle count of the radula is usually taken as a distinguishing character, specific by some authors, generic by others. Size, color and sculpturing of the shell and the design of the operculum are other characters that have been considered important. Thus, of the snails assumed to be the intermediate hosts of *Schistosoma japonicum* in China, Bartsch (1936a, 1936b, 1939, 1946) recognized, mainly on basis of denticle count, 16 species belonging to 3 genera, namely, *Oncomelania, Katayama* and *Schistosomophora.* The 16 species with the differences as considered by Bartsch are listed in Table 1. Annandale (1924) in "The molluscan hosts of the human blood fluke in China and Japan, and species liable to be confused with them" suggested a single genus for the schistosome carrying snails, with two sections using the presence or absence of axial ribs on the shell as the differential character. The same character was accepted by Bequaert (1934) and Pilsbry (1934) to distinguish *Oncomelania* from *Blandfordia* (= *Katayama*). But on the other hand, Chen and Li (1930), Li (1932, 1934), Wu (1941), Wu and Hsu (1942) and Abbott (1945, 1946) have suggested that neither the radular formula nor the character of the shell constitutes a reliable criterion for the classification of these small snails.

Since this disagreement exists, it is considered appropriate to present certain information which we have accumulated concerning these characters. In September–October 1946 one of us (CPM) during an epidemiological survey in Soochow-Wusih area with Mr. C. C. Wu, parasitologist of the National Institute of Health, Nanking, collected a certain number of the snail-hosts of *Schistosoma japonicum* from 7 villages, all of them situated within an area of 40 miles across. The radular formula and shell character of these snails were studied intensively in an attempt to evaluate their usefulness in taxonomy.

Technique and methods. Only mature snails with intact shells were used. The following data were obtained from each snail: 1) number of whorls, 2) length and width, 3) number of axial ribs for the three last whorls, except when the shell was smooth, and 4) radular pattern. The measurements were made with a caliper to the nearest tenth of a millimeter. The axial ribs were counted with the help of a hand lens. The radula was dissected out and treated with 20 per cent potassium

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hydroxide until free from tissue. It was then transferred to a drop of melted glycerin jelly on a slide. The plates and teeth were slightly teased apart under a dissecting microscope. A cover slip was put on, the whole specimen examined from one end to another under a high power microscope and all denticles on every tooth counted and tabulated. When denticle variations were observed within the same radula, for the sake of brevity in recording the counts, the number first observed was noted followed by variants in parentheses, regardless of the number of

TABLE 1.—*Snail-hosts of* Schistosoma japonicum *in China. (After Bartsch)*

Species	Localities	Radular formula	Other characters
1. *Oncomelania hupensis* Gredler, 1881	Wuchang, Hankow Huangchow	$\frac{1\ 1\text{–}1}{2\text{–}2}$; 2–1–3 ; 7 ; 5	Average ribs on the last whorl : 12
2. *O. schmackeri* (von Moellendorff, 1888)	Kating, Sunkiang, Wukiang, Soochow, Shanghai, Kunshan, Nanking, Chinkiang, Kashing	ditto	Average ribs on the last whorl : 14.1
3. *O. mollendorffi* (Schmacker & Bottger, 1890)	Kashing, Kashan, Puchen, Ninkuo	$\frac{1\text{–}1\ \ 1}{2\ \ 2}$; 2–1–3 ; 9 ; 5	Average ribs on the last whorl : 19
4. *O. longiscata* (Heude, 1890)	Yoyang	ditto	Average ribs on the last whorl : 12.6
5. *O. crassa* (Heude, 1890)	Tunglieu	?	Periostracum greenish black Intercostal spaces wide
6. *O. costulata* (Heude, 1890)	Chinchow	?	Axial ribs more numerous than previous species
7. *O. elongata* Bartsch, 1936	Chekiang Province	$\frac{1\text{–}1\text{–}1}{2\text{–}2}$; 2 -1 -3 ; 7 ; 5	More whorls Average ribs on the last whorl : 15
8. *O. multicosta* Bartsch, 1936	Hanchow, Wuhing	$\frac{1\ \ 1\ \ 1}{2\ \ 2}$; 2–1–3 ; 6 ; 5	Average ribs on the last whorl : 20
9. *O. yaoi* Bartsch, 1939	Pingyang	$\frac{1\text{–}1\text{–}1}{2\text{–}2}$; 2–1–3 ; 8 ; 5	Brilliant translucent wax color with pinkish early whorls, axial ribs distantly spaced
10. *Katayama formosana* (Pilsbry & Hirase, 1906)	Formosa	$\frac{1\text{–}1\ \ 1}{3\text{–}3}$; 2–1–3 ; 9 ; 5	Elongate ovate shape
11. *K. fausti* Bartsch, 1925	Shaohing	$\frac{1\ \ 1\ \ 1}{3\ \ 3}$; 2–1–3 ; 8 ; 5	Size smaller than *K. lii* Shell heavier and coarser than *K. cantoni*
12. *K. cantoni* Bartsch, 1925	Fatshan	$\frac{1\ \ 1\ \ 1}{3\ \ 3}$; 2–1–3 ; 7 ; 5	Shell thinner and more fragile than *K. fausti*
13. *K. lii* Bartsch, 1936	Linan, Siaofeng	$\frac{1\ \ 1\ \ 1}{3\ \ 3}$; 2–1–3 ; 9 ; 7	Size larger than other Chinese species
14. *K. tangi* Bartsch, 1936	Futsin	$\frac{1\text{–}1\text{–}1}{3\text{–}3}$; 2–1–3 ; 9 ; 5	Slightly larger than *K. formosana* Shell pinkish
15. *Schistosomophora slateri* Bartsch, 1946	Penhsien	$\frac{2\text{–}1\text{–}2}{3\text{–}3}$; 2–1–3(4) ; 11 ; 6(7)	
16. *S. robertsoni* Bartsch, 1946	Tali	$\frac{2\text{–}1\text{–}2}{2\ \ 2}$; 2 -1–3 ; 8 ; 5	

consecutive rows showing such variation. Thus, when the variations in the upper edge of the rachidians were: 1–1–2, next 2–1–1, next 2–1–2 and finally 2–1–1, the abbreviated expression: 1(2)–1–2(1) will be used. The same applies to the rest of radular teeth.

The snails collected from 7 villages are numbered in the following way:

A. Chin Shan Hsiang, Motoh, Soochow
B. Chien Chia Chan, Motoh, Soochow
C. Hsu Chaun, Soochow
D. Loh'm, Soochow

E. Feng Hsiang Tsung, Wusih
F. Wu Ho Hsiang, Wusih
G. Shih Tai Chiao, Wusih.

From each lot 20–23 snails were picked. Altogether 148 snails were examined. Space does not permit the publication of the data from all the seven areas, so the data from Wu Ho Hsiang alone are presented here in Table 2 as an illustration. The results of the examination of 148 snails are compiled in Table 3. The numerous variations found on a single ribbon prohibit inclusion in this table. The formulae found in the majority of rows is therefore considered as the type for the entire ribbon. Furthermore, we have compared the radulae of 50 snails harboring

TABLE 2.—*F: Wu Ho Hsiang, Wusih*

	No. whorls	Length	Width	L./W.	No. axial ribs			Radular formula
					ultimate	2	3	
1.	6.0	6.0	2.7	1 : 2.22	16	16	16	$\frac{1(2)-1-1(2)}{2-2}$; 2–1–3(4) ; 8 ; 5
2.	5.9	6.0	2.6	1 : 2.31	20	17	17	$\frac{1-1-1}{2-2}$; 3–1–3 ; 9 ; 6
3.	6.0	6.2	2.6	1 : 2.38	18	18	16	$\frac{1-1-1}{2-2}$; 2–1–3 ; 8 ; 5
4.	5.6	6.3	2.7	1 : 2.33	19	19	19	$\frac{1-1-1(2)}{2-2}$; 2–1–3 ; 9 ; 6
5.	5.5	6.3	2.9	1 : 2.17	22	..	..	$\frac{1(2)-1-1(2)}{2-2}$; 2(1)–1–3(5,4) ; 9 ; 6
6.	5.7	6.3	2.7	1 : 2.33	20	20	..	$\frac{1(2)-1-1}{2-2}$; 2–1–3 ; 8(9) ; 5
7.	5.2	6.3	2.7	1 : 2.33	20	20	20	$\frac{1-1-1(2)}{2-2}$; 2–1–5 ; 8 ; 5
8.	6.0	6.4	2.9	1 : 2.2	18	18	18	$\frac{1(2)-1-1(2,3)}{2-2}$; 2–1–3(4) ; 8 ; 5
9.	5.8	6.4	2.8	1 : 2.38	..	19	..	$\frac{1(2)-1-1(2)}{3-3}$; 2(3)–1–3 ; 9 ; 6
10.	6.4	6.5	2.6	1 : 2.5	19	18	19	$\frac{2(1)-1-1(2)}{2-2}$; 2–1–3 ; 8 ; 6
11.	6.3	6.5	2.5	1 : 2.6	19	20	..	$\frac{2(1)-1-1(2)}{2-2}$; 2–1–4 ; 8 ; 5
12.	6.2	6.6	2.9	1 : 2.27	..	20	..	$\frac{1(2)-1-1(2)}{3-3}$; 2–1–3(4) ; 8 ; 4
13.	6.8	6.8	2.7	1 : 2.51	20	17	17	$\frac{1-1-1}{2-2}$; 2–1–3 ; 7 ; 4
14.	5.8	6.8	2.7	1 : 2.51	19	20	19	$\frac{1-1-1}{2-2}$; 2–1–1(3) ; 8 ; 6
15.	6.3	6.9	2.9	1 : 2.37	19	22	17	$\frac{1(2)-1-1(2)}{3-3}$; 2–1–2(3,4) ; 7 ; 5
16.	5.7	6.9	2.9	1 : 2.38	16	16	16	$\frac{1(2)-1-1}{2-2}$; 2(1)–1–3(4) ; 7 ; 4
17.	6.3	6.9	2.8	1 : 2.46	19	20	18	$\frac{1(2)-1-2(1)}{2-2}$; 2–1–3(4,5) ; 9 ; 6
18.	6.0	6.9	2.8	1 : 2.46	22	23	..	$\frac{2(1)-1-1(2)}{2-2}$; 2(3)–1–3(4,2) ; 8 ; 5
19.	6.0	7.0	2.8	1 : 2.5	18	18	17	$\frac{1(2)-1-1(2)}{2-2}$; 2–1–3(4) ; 8 ; 5
20.	5.9	7.0	2.7	1 : 2.59	19	22	19	$\frac{1-1-1(2)}{3-3}$; 2–1–3(4) ; 8 ; 5
21.	5.9	7.0	2.9	1 : 2.44	22	19	..	$\frac{1(2)-1-1(2)}{3-3}$; 2–1–3(2) ; 8 ; 5
22.	6.4	7.8	2.8	1 : 2.78	20	21	20	$\frac{1-1-1(2)}{3-3}$; 2–1–3(4,2) ; 8 ; 5
23.	7.2	7.9	2.8	1 : 2.82	18	18	19	$\frac{1(2)-1-1(2)}{2-2}$; 2–1–3(4,5) ; 8 ; 5
Aver.	6.0	6.68	2.75	1 : 2.43	18.6	18.7	18.0	

schistosome cercariae and 50 uninfected snails, both collected from Chin Shan Hsiang and found that the denticle count in one group showed no significant difference from that of the other.

Discussion. We believe that this is the first time that data from these snails have been so secured that detailed comparisons are possible. It would be observed from our data that:

1) In snails collected from a given locality the radular denticle count varies greatly from one snail to another and also within the same snail.

2) There is no correlation between the radular characters and an infection with *S. japonicum* snails collected from a same locality.

TABLE 3.—*Résumé of the characters of 148 snails from 7 villages*

A. *Shell characters*

	Minimum	Maximum	Average
Number of whorls	4.6	7.8	6.35
Length	5.8	9.0	7.51 mm
Width	2.5	4.0	2.97 mm
Number of axial ribs			
Ultimate	12	27	17.1
2	12	26	16.9
3	12	25	16.6

B. *Radular pattern*

Rachidian	No. snails	Percentage	*Lateral*	No. snails	Percentage
1–1–1 / 2–2	91	61.5	2–1–3	106	71.6
			2–1–2	17	11.5
			1–1–2	8	5.4
1–1–1 / 3–3	35	23.6	2–1–4	7	4.7
			1–1–3	4	2.7
			2–1–5	2	1.4
2–1–1 / 2–2	7	4.7	others	4	2.7
			Inner marginal		
2–1–1 / 3–3	4	2.7	8	98	66.2
			7	25	16.9
			9	18	12.2
2–1–2 / 3–3	3	2.0	6	4	2.7
			10	3	2.0
2–1–2 / 2–2	2	1.4	*Outer marginal*		
			5	97	65.5
			4	38	25.7
Others	6	4.0	6	13	8.8

3) There is no correlation between: a) number of whorls, or b) the size of the shell, or c) the number of axial ribs, or the smoothness of the shell with the radular denticle count.

We have tried to extract from each lot snails having the same number of whorls, or the same sculpturing and make comparisons, but failed to find any indication that a group could be formed with one or several of these characters in common. Some similar features are found only in 3 snails having basal denticles 4–4. They have the same number of whorls, inner and outer marginal denticles and their size not greatly different. But the rachidian part is differently cusped, and most interesting of all is the shell which in one is strongly costate, in another is only slightly so, and in the third is entirely smooth.

Most Chinese authors working on schistosomiasis have observed such variability in the number of cusps. However, they have either stated such findings in a few words or merely by verbal communications without making special emphasis. Taking differences in denticle count as criteria for the classification and considering only those snails with a uniform count throughout the whole radula, we would have in our material just presented at least 10 species out of 16 accepted by Bartsch.

It is to be doubted that so many could occur in the same area sharing the same bionomics.

It is concluded that the denticle pattern cannot be relied upon for taxonomic purposes in this group of snails. It may be pointed out that this has been demonstrated previously also in *Viviparus* (van Cleave and Richey, 1936) and some members of the family Pleuroceridae (Howe, 1938). Abbott's recent work (1945, 1946) on *Oncomelania quadrasi* (*Schistosomophora quadrasi*) of Philippine Islands leads him to the conclusion that we are quoting here: "It is evidently unsound to create species or genera, at least in this group, on the basis of denticle counts which vary as much as they do."

It is very doubtful that the smoothness of shell could be taken as a distinguishing character among snails of this group. Chen and Li (1930) and Li (1934) have observed that the nearer to the hilly regions, the thinner and smoother the shell. In our series, snails of batch A have comparatively smooth shells, as indicated by the less distantly spaced axial ribs. They were collected from a village situated at a hill base. As a matter of fact, the name of the village, Chin Shan Hsiang, means Golden Hill Village. It is of interest to note the topography of areas where snails listed in Table 1 were recorded. In the group *Oncomelania,* the alleged species *O. hupensis, O. longiscata, O. costulata,* all of them having strongly costate shells, were collected from lake regions. The moderately costate species *O. schumackeri, O. moellendorffi, O. elongata* and *O. multicosta* were from more hilly regions, as were the supposed members of the group *Katayama,* which have a smooth shell. Most interesting are the 2 species of *Schistosomophora* recently described from China that have a very smooth shell. *S. slateri* is found in Penhsien, Szechwan, at about 3,300 feet and *S. robertsoni* in Tali, Yunnan, at about 6,900 ft. above sea level.

The number of whorls can be taken into account only when full grown adult snails are examined. As the snails grow whorl by whorl, it is obvious that young snails will have a smaller number of whorls than the adults. Since the complete growth requires 5 months and hibernation 3–4 months (Li, 1934), it is most likely that each collection will contain a fair proportion of snails of less than full growth.

The color of the shell is sometimes used but varies with the environmental conditions. It is not infrequent to find living snails that have been kept dried for some time with their shells covered with algae, giving them a dark green color. In December, 1946, a batch of snails collected at Kashing, Chekiang was sent to Dr. H. E. Meleney in New York where they were kept living in an aerated aquarium. In August 1947, another shipment of snails collected from the same area was delivered. One can hardly believe that the 2 batches were from the same locality. Those of the first shipment were clean, with pinkish apex while those of the second lot were dark greenish.

It is not suggested that all the snail-hosts of *Schistosoma japonicum* in China are of the same species, but we do believe that a better basis must be found for separating them into species than radular formula, sculpturing, or the color of the shell.

Summary. One hundred and forty-eight oncomelaniid snails known to be capable of transmitting oriental schistosomiasis which were collected from 7 villages

near Soochow-Wusih, Kiangsu, China, were subjected to individual observation regarding the number of whorls, length and width, number of axial ribs for the last three whorls and denticle count of the radular teeth. It was found that the denticle count varies to such an extent that it is not a dependable criterion for separating species or genus in this group. The same conclusion is suggested for shell characters.

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MYRMONYSSUS CHAPMANI, A NEW SPECIES OF HYPOASPID MITE (ACARINA: LAELAPTIDAE)

EDWARD W. BAKER[1] AND R. W. STRANDTMANN[2]

The mite to be described is of interest taxonomically, because it is structurally so heterogeneous that its reference to any of the known subfamilies of LAELAPTIDAE is not completely satisfactory.

In 1941 Vitzthum proposed thirteen subfamilies for the LAELAPTIDAE and placed *Myrmonyssus* into the first of these, the HYPOASPIDINAE. This subfamily he characterized as follows: "Tarsi I with pretarsus, claws and pulvillus (except in *Anystipalpus* and *Laelantennus*). In the myrio- and myrmeco-philes the claws are frequently recurved or entirely lacking. The female genital plate is drop-shaped and more or less broadened behind coxae IV and may extend close to or be widely separated from the triangular anal plate. The genital plate possesses only one pair of setae although the number may be increased if the ventrals unite into a genitoventral plate. Sometimes (*Ololaelaps* and *Stylochirus*) the genitoventral plate is confluent with the anal plate. The chelicerae have normal, strong chelae but in some myrmecophiles these chelae are weak and abnormally placed. The epistome is a simple or serrate convex line."

As a rule, subfamily divisions are rather artificial; rarely is it possible to make clear-cut distinctions in this category. We feel that in many respects Vitzthum's classification is in error but nevertheless it is a long step toward the final organization of the mites.

It does appear as if Vitzthum used the subfamily HYPOASPIDINAE as a catch-all, as evidenced by the long list of genera and subgenera he placed in it and by the fact that Trägardh in 1946 withdrew *Passalobia* Lombardini and placed it into an entirely different cohort. However, because of the paucity of knowledge on the mites it is probably best to keep *Myrmonyssus* in the HYPOASPIDINAE until a more thorough study of the group can be made.

At first sight this new species of *Myrmonyssus* appears to belong to the parasitic LIPONYSSINAE as evidenced by the epistome, the untoothed chelicerae and the genitoventral plate. However, it differs from the LIPONYSSINAE in the absence of claws on the tarsi, in lacking a pronounced spur on the anterior margin of coxa II, in the shortness of the rounded fixed chela, in the type of hypostomal dentation and in the presence of the five anal setae and the paired setae on coxa IV.

Myrmonyssus Berlese 1903

The following characters appear to be distinctive of this genus: Fixed chela of chelicerae weakly developed, blunt; both chelae without teeth; dorsal shield large, blunt on rear, covering all but the posterior portion of body; anal plate on rear of mite; coxa I–IV each with a pair of setae; tarsal claws lacking; genito-ventral plate pointed on rear, not reaching anal plate.

Type: Myrmonyssus diplogenius Berlese 1903, collected in ants' nests, Italy.

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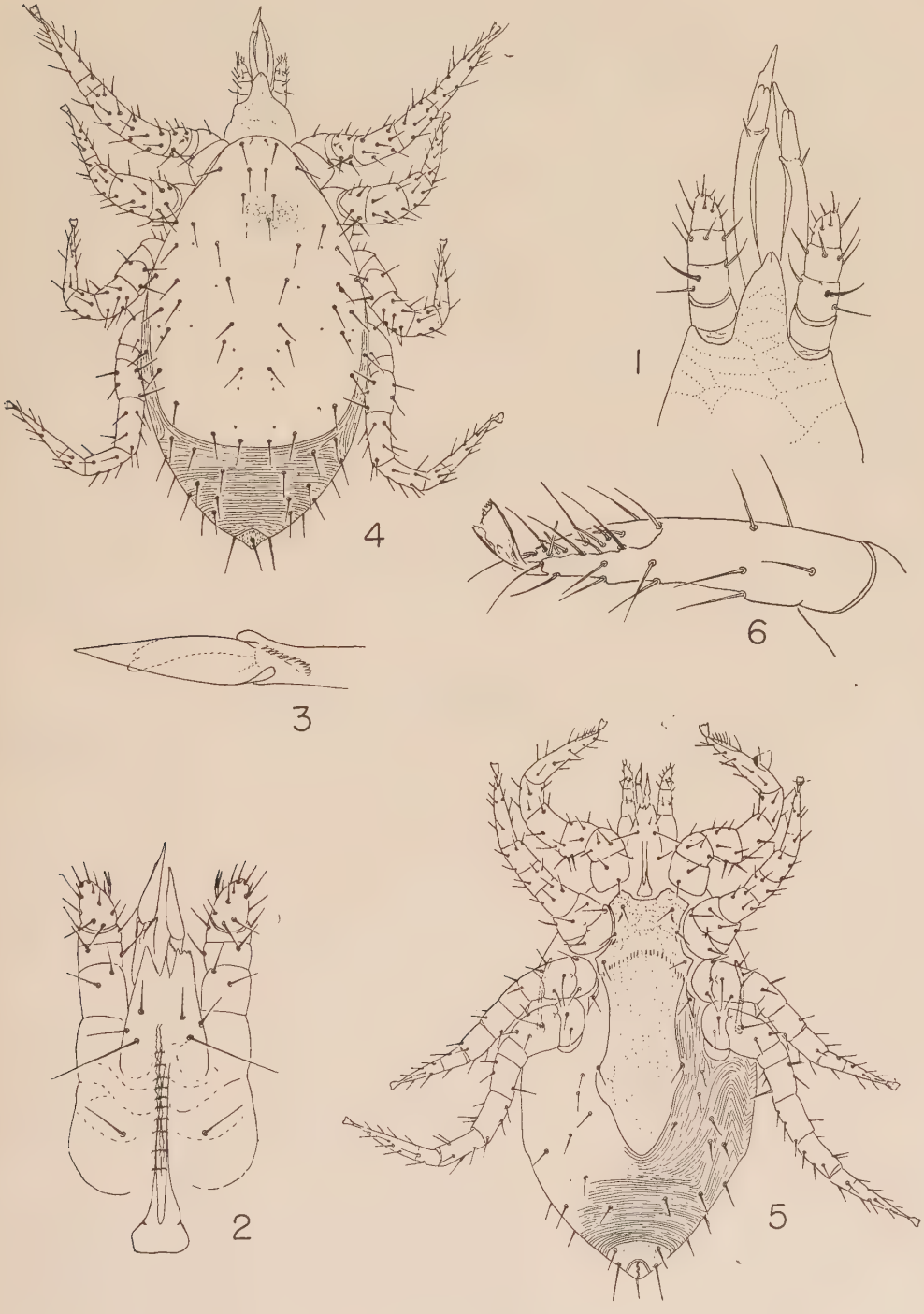
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Myrmonyssus chapmani, new species

Female (Figs. 1–6) : Palpus relatively short with a few strong setae (Fig. 1). Chelicerae slender; below base of fixed chela a small spinelike seta (Figs. 2, 3) ; below base of movable chela a row of small teeth; fixed chela weakly developed, bilobed, not pointed distally and shorter than the pointed movable chela. Epistome reticulate, elongate, covering part of gnathosoma. Hypostome with at least ten transverse rows of minute teeth, about seven or eight teeth per row. Dorsal shield (Fig. 4) large, blunt covering all but a small posterior portion of the mite, with twenty-three pairs of setae. Rest of dorsum striated, bearing setae as figured. Anal plate with five setae, located on posterior margin of mite, the scobinate posterior portion bearing the unpaired seta situated dorsally, the anal opening and paired setae ventral (Fig. 5). Sternal plate large, as long as wide, bearing the usual three pairs of setae; the posterior portion of the plate is difficult to distinguish but appears to be patterned and not strongly arched. The genitoventral plate with one pair of setae, patterned and shaped as in figure 5. No metapodal plates. Coxae I–IV each with a pair of setae; no strong tooth or spur on anteriordorsal portion of coxa II. Peritreme short, not extending past anterior margin of coxa III. Legs long, of medium strength, with setae as shown in Figs. 4 and 5. All tarsi lacking claws but with tarsal suckers (Fig. 6). Length of body, not including gnathosoma, 657 μ; width 385 μ (type) ; paratype 685 μ long by 385 μ wide.

Male and immature forms not known.

The type and paratype were collected at Laredo, Texas, September 2, 1947, on orchid plants originating in Mexico, D. F., by T. P. Chapman for whom the mite is named.

The new species differs from *Myrmonyssus diplogenius* Berlese 1903 in the shape of the anal plate, in the number of anal setae; in having a longer peritreme, and in having a bilobed fixed chela.

From the relationships of the mite it appears to be myrmecophilous, but this is, of course, conjectural.

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THE RESISTANCE OF EGG-LAYING DUCKS TO INFECTION BY THE MALARIA PARASITE *PLASMODIUM LOPHURAE*

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Ducks have been generally considered to be highly susceptible, regardless of age and sex, to infection by the erythrocytic forms of *Plasmodium lophurae* (Wolfson, 1940; Hewitt, 1942). It was therefore surprising to find in 1942 several adult ducks which failed to develop any appreciable infection in spite of the fact that they had been inoculated intravenously with a large dose of heavily parasitized blood from ducklings. In the four resistant individuals which were first observed the parasites persisted in low numbers (50 to 200 per 10,000 red blood cells) for 3 weeks after inoculation. In smears made approximately every third day during this period, parasites of abnormal appearance became noticeable 3 to 10 days after inoculation and thereafter formed a progressively larger proportion of the small number of parasites present. Very few parasites with over two nuclei were ever seen, the very young trophozoites soon disappeared, and one to two nucleate trophozoites of normal appearance became less and less abundant. The abnormal forms were large and of two types, which may briefly be called "Dark" and "Light." The "Dark" forms showed after staining with Giemsa a dense blue cytoplasm, quite unlike the delicate blue usually seen in normal parasites. They had a large deep-pink nuclear mass. The "Light" forms showed a diffuse pale pinkish stain, suggesting a cell about to disintegrate. Although these two forms might at first be mistaken for female and male gametocytes, their pigment granules were small rather than coarse, as they are in the gametocytes of *P. lophurae*. The impression given by the blood smears was that in these 4 ducks the multiplication of the parasites was strongly inhibited. It soon became apparent that age was not the important factor, for several ducks even older than the 4 resistant ones were found to be fully susceptible. The observations were interrupted by the author's service in the Army. They were resumed in 1946 using a different strain of *P. lophurae* (obtained through the kindness of Dr. A. P. Richardson). With this strain an exact repetition of the earlier results has not been obtained, since the ducks used proved to be either highly susceptible or so resistant that they soon eliminated the infection, so that very few of the abnormal parasites were seen. It has, however, been possible to correlate resistance with egg-laying activity.

METHODS

The strain of parasites was maintained at a high level by weekly intravenous passage in ducklings one to two weeks old.

The ducks were all White Pekins and were obtained as day-old ducklings. They were reared indoors until 6 to 8 weeks old, and thereafter in an outdoor pen with a screen bottom. They were fed a mixture of 50% ground whole wheat, 25% meat scrap, and 25% ground yellow corn. This was supplemented twice a week

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with lettuce or some other green vegetable. Some, but not all, of the female ducks began laying eggs when they were 6 to 7 months old. As groups of 3 to 5 ducks reached this age, they were placed in individual indoor cages where their egg production could be readily followed. A heparinized blood sample (2 ml) was taken from some of the ducks and the plasma prepared for assay for its content of biotin and bound fat-soluble biotin-active material (Trager, 1947). The ducks were then

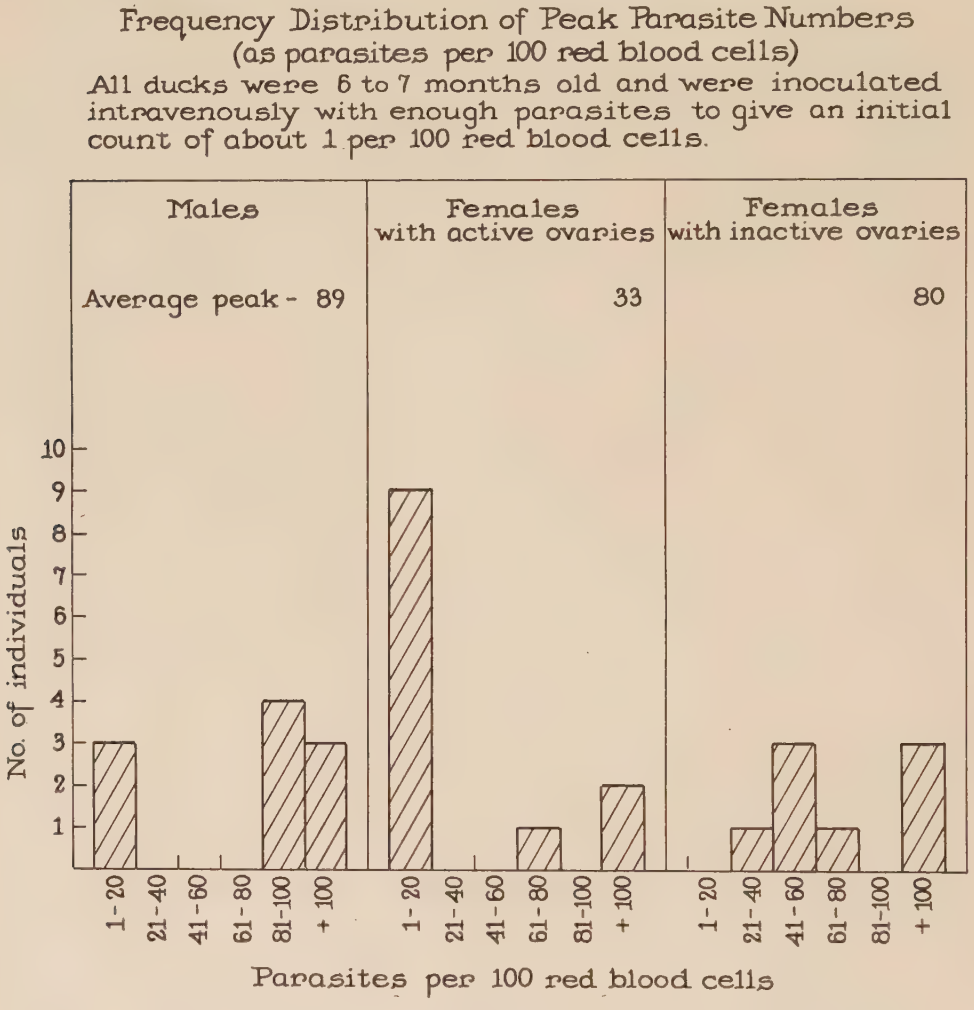
Frequency Distribution of Peak Parasite Numbers
(as parasites per 100 red blood cells)
All ducks were 6 to 7 months old and were inoculated intravenously with enough parasites to give an initial count of about 1 per 100 red blood cells.
Males
Females with active ovaries
Females with inactive ovaries
Average peak - 89
33
80
No. of individuals
1 2 3 4 5 6 7 8 9 10
1-20 21-40 41-60 61-80 81-100 + 100
Parasites per 100 red blood cells

FIG. 1. A comparison of the density of parasitemia attained in female ducks with active and inactive ovaries and in males inoculated with the erythrocytic forms of *P. lophurae*.

inoculated intravenously with a dose proportionate to their body weight of heavily parasitized blood from an infected duckling. Enough parasites (one to 1.5 billion per kilogram body weight) were injected to give an initial parasite density of approximately one per 100 erythrocytes. Young ducklings, similarly inoculated as controls, invariably developed heavy infections. A thin blood smear, stained with Giemsa, was prepared from each duck within a minute after inoculation and once daily thereafter until death or recovery. These smears were used to determine the numbers of parasites per 100 red cells.

Birds which survived the infection were killed, and these, as well as the birds which died, were autopsied to confirm their sex, as determined by external characters, and to note the state of development of the ovary and oviduct or the testes.

RESULTS

Ten male and twenty female ducks were inoculated and observed in the manner described in the preceding section. The results are shown in Fig. 1. Of the 10 males, 7 developed very heavy infections, with peaks of over 81 parasites per 100 red blood cells. On the average, the peak parasite number occurred on the fifth day after inoculation. Among the females, 12 had active ovaries as shown either by the fact that they laid eggs shortly before inoculation or that, at autopsy, they showed a large oviduct and an ovary containing numerous developing yolks. The other 8 females did not lay eggs and, when autopsied, showed a small oviduct and a small ovary with no developing yolks. Of the 12 females with active ovaries, 9 had light infections, with a peak parasite number of 20 or less. In several of these the parasites underwent no increase in number whatever, so that the peak parasite number was the number present immediately after inoculation. The average time to reach the peak parasite number was only 3.4 days. In these resistant ducks, the phenomenon of long persistence of small numbers of parasites, consisting chiefly of abnormal forms, was not encountered. A few abnormal forms were seen, but in all the resistant birds the infections were rapidly cleared out. Of the 8 females with inactive ovaries 5 developed moderately heavy infections and the other 3 very heavy infections. The average peak parasite number was about the same as for the males. Similarly, the average time to the peak parasite density was 5.4 days.

TABLE 1.—*The concentrations of biotin and bound fat-soluble biotin-active material (FSF) in the plasma of 6 to 7 month old ducks*

Type of duck	No. of animals assayed	Biotin mγ/ml		Bound FSF as mγ of biotin activity per ml	
		Range	Average	Range	Average
Male	7	5–13	7	18–21	19
Female with active ovary	9	13–32	25	20–39	29
Female with inactive ovary	4	9–25	17	18–24	21

The plasma concentrations of biotin and of the bound fat-soluble material, expressed in terms of its biotin activity, are shown in Table 1. It is evident that both the biotin and the lipoprotein (source of the bound fat-soluble material) were higher in the plasma of female ducks than in that of males, and that both were higher in the plasma of females with active ovaries than in that of females with inactive ovaries.

DISCUSSION

There can be no doubt that some physiological characteristic ordinarily associated with egg formation in the duck is responsible for the transformation of this animal otherwise highly susceptible to infection by *P. lophurae* into one highly resistant. The few females with active ovaries which nevertheless developed heavy infections, and the few resistant males, serve to emphasize the fact that individual differences in susceptibility occur which are quite unrelated to egg formation. In

most of the ducks, however, these individual variations are overshadowed by the characteristic associated with egg formation.

Other work (Trager, 1947) has indicated that a plasma lipoprotein plays a role in the resistance of birds to avian malaria parasites. In keeping with this hypothesis is the fact that the lipoprotein content of the plasma of ducks with active ovaries is higher than that of ducks with inactive ovaries. Riddle and Burns (1927) have previously shown a much higher concentration of lipides in the blood of female ring doves during the ovulation cycle than in males, or females in the resting stage. Lorenz, Entenman and Chaikoff (1938) have demonstrated the same situation in more detailed fashion for the chicken. They have further shown that the increase in lipides can be produced by the injection, into immature male as well as female birds, of estrogenic hormones (Entenman, Lorenz and Chaikoff, 1940). These facts suggest the possibility of an indirect hormonal effect, via the lipoprotein of the plasma, on resistance to *P. lophurae.* It is worthy of note that estradiol benzoate has been observed to produce a mild lipemia in rats on a diet high in fat (Loeb, 1942), and that urinary estrogens increased the bactericidal effects of the blood serum of male rabbits on *Eberthella typhi* (Tsao, 1941).

Bennison and Coatney (1948) have recently reported a small effect of sex of the host on *Plasmodium gallinaceum* infection in very young chicks. Female chicks 6 to 8 days old showed slightly higher densities of erythrocytic parasites, a poorer response to treatment with quinine, and earlier appearance of exoerythrocytic forms in the brain than did males. A more striking effect of sex on the course of a protozoan infection, and one perhaps more nearly analogous to the results reported in the present paper, is that described by Hauschka (1947) for *Trypanosoma cruzi* in mice. When brother and sister mice were inoculated in a comparable manner with *T. cruzi* the infection in the males attained parasite densities twice as great as those seen in the females.

SUMMARY

Ten male and twenty female White Pekin ducks 6 to 7 months old were inoculated intravenously with large doses of *Plasmodium lophurae.* In 9 of the 12 female ducks with active ovaries at the time of infection, the parasites underwent little or no multiplication. All 8 of the females with inactive ovaries developed heavy infections, as did 7 of the 10 males. The average free biotin and bound biotin-active lipoid material of the plasma before inoculation were highest in the egg-laying females, lowest in the males and intermediate in the females with inactive ovaries.

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EFFECTS OF DIGESTION AND REFRIGERATION ON THE ABILITY OF *TRICHINELLA SPIRALIS* TO INFECT RATS

Otto F. Gursch*

It is the common procedure in this laboratory to obtain trichina larvae by artificial digestion of infected muscles and to store the decapsulated larvae at a temperature of 5° C until used for actual experimental work. This temperature preserves the vitality of such larvae over a longer period of time than would ordinary room temperature. The question then arose in regard to whether or not different periods of artificial digestion and exposure to different periods of 5° C temperature might alter the capacity of these larvae to infect susceptible animals.

The trichina larvae used for this experiment were obtained from infected stock rats which were skinned, eviscerated, ground up and soaked in tap water overnight

Table 1.—*Recovery of* Trichinella spiralis *from the digestive system of experimentally infected rats with larvae previously exposed to 4 to 12 hours of artificial digestion and 0 to 72 hours of refrigeration**

Rat	Wt. in grams	Hrs. of digest.	Hrs. of refrig.	No. of larvae given	No. of worms recov.	Percent recovered
1	250	4	0	3000	2053	68.4
2	225	8	0	3000	1837	61.2
3	240	12	0	3000	1283	42.8
4	195	4	24	3000	1895	63.2
5	195	8	24	3000	1802	60.1
6	235	12	24	3000	1513	50.4
7	225	4	48	3000	1901	63.4
8	200	8	48	3000	1916	63.9
9	180	12	48	3000	died	
10	215	4	72	3000	1190	39.7
11	215	8	72	3000	854	28.5
12	200	12	72	3000	1106	36.9
13	222	4	0	5000	2531	50.6
14	225	8	0	5000	2303	46.1
15	210	12	0	5000	1687	33.7
16	220	4	24	5000	2404	48.1
17	213	8	24	5000	2337	46.7
18	210	12	24	5000	2493	49.9
19	193	4	48	5000	1883	37.7
20	162	8	48	5000	2054	41.8
21	150	12	48	5000	1107	22.1
22	197	4	72	5000	805	16.1
23	195	8	72	5000	990	19.8
24	170	12	72	5000	611	12.2

* The rats were killed 24 hours after they were infected.

in the refrigerator. On the following morning the ground-up rat tissue was mixed with a solution of 1% pepsin and 0.5% of hydrochloric acid (1:80). This mixture was then divided into 3 parts and immersed in a water bath at a constant temperature of 37° C. Air was used to agitate the solution continuously. After digestion for 4, 8 and 12 hour periods respectively, the decapsulated larvae were washed free of undigested material in warm tap water. From the larvae of each digestion period, doses of 3000 larvae were prepared by dilution count. Immediately 3 of these doses were given by stomach tube to anesthetized rats, while the remaining 3000 larvae doses were kept in large centrifuge tubes with tap water and subjected to 24, 48 and 72 hours of refrigeration at 5° C before being used for in-

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fections. The infected animals were left with water but without food, and killed after 24 hours, which allowed sufficient time for the larvae to burrow into the mucosa. The entire procedure was repeated with a set of rats infected with doses of 5000 larvae.

At autopsy, the small intestine of each rat was removed, slit open and placed in 0.85% saline solution which was kept at a temperature of approximately 37° C. Subsequently it was incubated for 1 hour at 37° C before the mucosa was stripped from the muscularis and broken up by vigorous shaking, then examined for worms. The stomach, large intestine and cecum were each treated similarly. Any fecal material found here was also carefully examined for parasites.

The number of worms recovered from the entire gut was tabulated according to the period of refrigeration (Table 1). The total percentage of trichina larvae recovered from each rat indicated that 4 to 8 hours of digestion and up to 24 hours storage in the refrigerator did not impair the infectivity of the larvae. It is apparent that a 12 hour period of digestion resulted in a lowering of the percentage of worms recovered. Exposure to 72 hours of refrigeration was definitely injurious to the larvae even in combination with few hours of digestion, as seen in Table 1.

THREE NEW SPECIES OF CULEX (DIPTERA: CULICIDAE) FROM COLOMBIA*

LLOYD E. ROZEBOOM[1] AND W. H. W. KOMP[2]

During a mosquito survey at Villavicencio, Colombia, during the summer of 1947, several new species were taken, of which three especially interesting *Culex* are described below.

Culex (*Culex*) *scutatus* new species

FEMALE. A medium-sized mosquito with dark legs and wings and a striking scutal coloration pattern which distinguishes it from most other species of the subgenus *Culex*. *Head:* Proboscis mostly dark but with a small to large area of pale scales at the middle on the undersurface; palpi dark, about one-sixth the length of the proboscis. Vertex with golden yellow narrow curved scales and upright forked scales of the same color; sides of occiput with some golden recumbent scales and dark upright forked scales posteriorly; eye margins with small flat whitish scales, a large area of broad flat white scales on each side. *Thorax:* Prothoracic lobe with at most a few pale scales centrally and several long setae. Upper region of posterior pronotum with a patch of narrow curved dark scales, sometimes with a few pale scales among the dark ones. Scutum (Fig. 1) clothed with small, narrow, dark, purplish-brown scales; against this background a pattern is formed by silvery scales arranged as a broad stripe along the antero-lateral margin, and also a sublateral spot or stripe internal and posterior to the anterior lateral markings; a few pale scales on the anterior promontory; a narrow bare dark stripe on each side of mid-line, the two converging posteriorly to the bare prescutellar area. Scutal and scutellar setae dark. Mid-lobe of scutellum with small, dark and pale scales. Pleural sclerites brown, with a pale area containing a patch of white scales across the middle portion of the mesepimeron and upper portion of the sternopleuron; the area bordering the sutures between the mesepimeron, meron, and lower sternopleuron also pale; a patch of white scales in the lower pale area of the sternopleuron. Lower mesepimeral bristle present. Wing scales dark. Anterior surface of fore coxae clothed with dark scales; this area of mid and hind coxae with white scales. Trochanter of fore leg with dark scales in front and pale scales laterally; trochanters of mid and hind legs with white scales. Fore femur dark scaled; mid femur mostly dark but white at base and with a white stripe on posterior surface broad at base and narrowing to the apex of the segment; hind femur mostly white but with a dark stripe on anterior surface which widens distally and encircles the apex of the segment. Tibiae and tarsi entirely dark except for a patch of short yellowish setae on the posterior surface, and some pale scales on the outer surface at the apex of the hind tibiae. *Abdomen:* Tergite I with a small central patch of dark scales and many long dark setae; tergites II–VIII dark scaled except for small lateral, basal white spots becoming progressively larger on distal segments. Sternites with basal white bands.

MALE. Coloration similar to that of the female, but the scutal markings may be more pronounced and form a more distinct lyre-shaped outline, with the sublateral stripes extending to the scutellum. Mid-lobe of scutellum pale-scaled centrally. Proboscis with a narrow pale ring at middle; palpal segment IV with a conspicuous patch of white scales at the base on ventral

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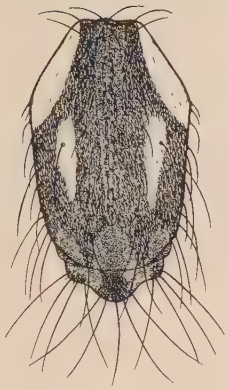
EXPLANATION OF PLATES

PLATE I

FIG. 1. *C. scutatus.* Scutum of adult.
FIG. 2. " " Sidepiece of male genitalia.
FIG. 3. " " Inner plate of mesosome.
FIG. 4. " " Ninth tergite of adult male.
FIG. 5. " " Head of larva.
FIG. 6. " " Mentum of larva.
FIG. 7. " " Terminal abdominal segments of larva.



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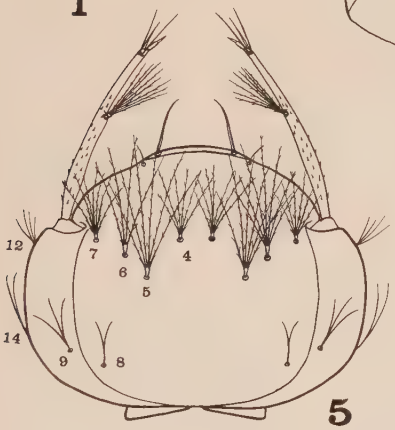

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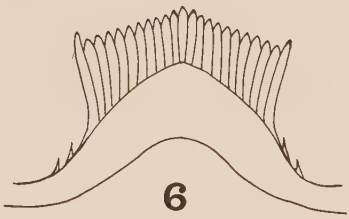

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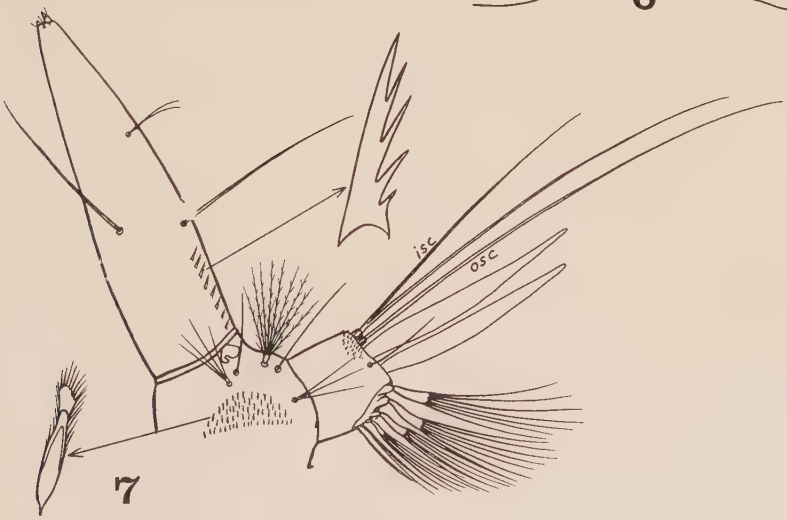

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surface; segment V with a few white scales at base. Abdominal tergites IV to VIII with basal pale bands which may be interrupted centrally. *Genitalia:* Sidepiece (Fig. 2) conical, about twice as long as wide, outer surface with several long setae, inner surface with short setae. Subapical lobe undivided, with two stout and one more slender rods below; upper angle of lobe slightly produced, with one, two or three stout hooked filaments; a long, slender leaf, exceeding the hooked filaments; and a slender curved seta arising from the outer surface of the lobe. Clasper a little over half the length of the sidepiece, somewhat swollen medially, distal third curved inwardly, tapering, with two minute setae before the tip; terminal spine broad and appendiculate. Inner plate of mesosome (Fig. 3) quadrate; a dozen or more stout denticles along the upper margin; upper (UA) and lower (LA) arms thumb-like; a long arm from base exceeding the plate, swollen medially and sharply narrowing to the tip, the inner margin sinuate and revolute. Tenth sternites tufted at tip with a dense group of spines which are broad and blunt on one side and gradually change to slender pointed ones on the other side; basal arm long and curved. Ninth tergites (Fig. 4) a narrow band arched into a lobe on each side, each lobe with a row of five or six stout evenly-spaced setae.

LARVA. *Head* (Figs. 5 and 6): Antennae shorter than the head, slightly swollen above the base and then tapering gradually to the apex, spined on proximal two-thirds; a multiple-haired tuft about two-thirds the distance from the base, the part beyond the tuft only slightly more slender; terminal seta rather stout, subterminal setae long and slender; papilla large, stout at base. Preclypeal spines long and slender. Frontal hairs 4, 5, 6, and 7 situated posterior to base of antennae; hair 4 unusually large, with five to nine branches; hairs 5, 6, and 7 large, with four to ten branches, hairs 8 and 9 one-to-four-branched. Subantennal hair 12 with three to five branches; hair 14 double or triple. Mentum with teeth equal in size, about a dozen on each side. *Thorax:* Skin glabrous. Prothoracic pleural group with the hairs as follows: one very long; two short, fine, and single; and one very short, double. Mesothoracic pleural hairs as follows: two long, single, one long three-or-four-branched, and one minute. Metathoracic pleurals as follows: one long single; one long five-branched; one short, fine, single; and one minute. *Abdomen* (Fig. 7): Skin glabrous. Long lateral hair 6 of segments I and II with two or three branches, this hair on segments III–VII single. Comb with about 40 scales in a triangular patch; each scale fringed laterally and at apex. Siphon about four times longer than width at base, somewhat swollen before the middle and then tapering to apex; with three pairs of hairs, the first long, single, and situated before the middle on the ventral surface, the second long, single, inserted laterally at about the middle of the siphon, the third small, fine, with two to four branches inserted on the ventral surface about a third of the distance from the apex. Terminal hairs minute. Pecten of about 10 teeth on the basal third of the siphon; each tooth with several strong denticles along ventral margin. Anal segment ringed by the plate; a patch of small spines before the insertion of the caudal hairs; inner submedian caudal hair (isc) long and split at base into two branches, outer hair (osc) long and single; lateral hair short, single; ventral brush comprised of about 6 multiple tufts; anal gills about twice the length of the segment, slender, tapering.

TYPES. *Holotype:* Male, with associated larval and pupal skins and mounted genitalia; deposited in U. S. National Museum. Paratypes: Three males and two females with associated larval and pupal skins; three males, one female, and three larvae, all unassociated. Paratypes deposited in the U. S. National Museum, the British Museum, and the Johns Hopkins School of Hygiene and Public Health. All material collected by L. E. Rozeboom from a small muddy hoofprint at Villavicencio, Colombia, on 15 May, 1947.

Taxonomic Discussion.—Culex scutatus belongs to the *salinarius-apicinus* series of Edwards (1932), which is characterized by the presence of the lower mesepimeral bristle and dark tarsi. In this series the females of *C. chidesteri* and *erythrothorax* have pale markings on the proboscis; in addition, the males of *dolosus* and *spinosus* have pale scales on the proboscis. *C. scutatus* is readily distinguished from these species by the scutal markings; this pattern, in fact, should distinguish it from all other American species of the subgenus *Culex* with the possible exception of *C. carcinoxenus* Castro (1932). It differs from this species in pale markings of the proboscis, but especially in the structure of the larva. Frontal hair 4 in *carcinoxenus* is small and single, while in *scutatus* it is large and multple. Hairs 5 and 6 are less heavily branched in *carcinoxenus*. The teeth of the mental plates of the two species differ, while the four pairs of ventral siphon tufts of *carcinoxenus* are

short and two- to four-branched, in contrast to the three pairs of hairs, two long, single, and one small multiple on the siphon of *scutatus*. The genitalia of *scutatus* differ from those of *carcinoxenus* in the structure of the clasper, the size and shape of the leaflet on the apical lobe, and in the number of denticles on the inner plate of the mesosome. *C. corniger* Theo. may have a similar scutal pattern, but is readily separated from *scutatus* by the banded tarsi.

Theobald (1903) describes a scutal pattern for *C. bilineatus* that is strongly suggestive of that of *scutatus*. Edwards (1932), who presumably examined the types, recognized Dyar's (1928) synonomy of *bilineatus* with *virgultus* Theobald 1901. *C. virgultus* differs from *scutatus* especially in the structure of the inner plate of the mesosome, which, according to Dyar, has only one large central tooth between the upper and lower arms in the former species.

C. pseudojanthinosoma was established by Senevet and Abonnenc (1946) upon characters of the adult female and the larva. The larva of this species appears to be very similar to that of *scutatus*. Unfortunately the authors do not describe frontal hair 4, but if it is as heavily branched as hairs 5, 6, and 7, it could not have been overlooked. In addition, the mentum of *pseudojanthinosoma* has a strong median tooth, while this tooth in *scutatus* is similar to the lateral ones. The siphon of *scutatus* is not swollen as markedly as that of *pseudojanthinosoma*. The scutal pattern of the two species differ considerably; that of *pseudojanthinosoma* consists of three longitudinal parallel lines.

Culex (*Isostomyia*) *bamborum* new species

Female. A small grey and brown mosquito with greenish plurae. *Head:* Proboscis about as long as the fore femur, enlarged on apical third, clothed with broad, flat, dark scales except for a few pale scales at base on underside. Palpus about one-ninth the length of the proboscis, dark-scaled. Clypeus brown, with a yellowish pubescence. Torus and first flagellar segment pale brown; the remaining segments of the antenna dark, with black whorl hairs and pale short hairs. Vertex with silvery narrow curved decumbent-scales and yellowish brown erect forked scales; a patch of broad flat white scales on the sides. *Thorax:* Prothoracic lobe greenish-grey, with a few pale scales and some long brown setae. Posterior pronotum green; a few narrow pale scales along upper margin. Scutum greyish-brown; three bare stripes extending the length of the scutum, one narrow median stripe, on either side of which is a somewhat broader submedian stripe; the scales very narrow centrally and somewhat broader on the sides, pale brown to white in color; a small patch of broader white scales before wing root; setae very long, dark brown. Mid-lobe of scutellum with a few narrow, curved pale scales; scutellar bristles dark brown. Pleurae green; one lower mesepimeral bristle present; a few pale scales along posterior margin of sternopleuron. Postnotum greyish brown. Knob of halter dark-scaled. Coxae green, with pale transparent flat scales on anterolateral surfaces; trochanters pale. Femora pale-scaled below, dark above, fore and mid femora also with a few pale scales at apex; all tibiae and tarsi dark. Wings dark. *Abdomen:* Tergites clothed with broad flat bronzy-brown scales centrally and some pale ones along lateral margin; venter pale scaled.

Male. Coloration similar to that of the female. Palpus very short, about one-ninth the length of the proboscis. *Genitalia:* Sidepiece (Fig. 8) a little over three times as long as the width at base, conical towards the apex, the outer surface with some very long, strong setae; shorter setae on inner surface; fine pilosity over entire surface, these hairs somewhat denser and longer at the apex. Lobe divided, the outer (upper) division somewhat stouter and shorter than the inner (lower) one. Outer division with the following appendages: midway on the stem, a small expanded striate leaf on the outer aspect and several broad flat filaments on the inner aspect; at the apex, a long curved filament with an expanded, hooked tip inserted on the lower angle, a slender pointed leaf inserted on the upper angle, and between these a spindle-shaped, short, leaf-like seta, and a stout pointed seta. Inner division a long, stout column, ending in two closely appressed rods with hooked tips, the upper inserted at the end of the column, and the lower nearer the base. A large boss below the inner division of the lobe, clothed with long fine setae inserted in prominent tubercles on the outer aspect, and with two or more stout filaments among the setae

near the upper angle, the outer filament longer and broad, and sinuate in certain aspects. Clasper rather broad, a little more than half the length of the sidepiece, strongly curved at the middle; the tip with a short, upturned horn; an appendiculate spine at apex; the outer surface of the distal third of the clasper with many closely set lamellae. Inner plate of mesosome (Fig. 9) with the central area roughly forming a parallelogram; dorsal arm long and expanded at tip; ventral arm short, curved; basal hook broad. In extreme lateral view this plate (Fig. 10) appears as a rather narrow stem with a long, sharp-pointed dorsal arm and a short broad pointed ventral arm. Tenth sternites with a comb-like row of 9–12 stout, blunt teeth. Ninth tergite (Fig. 11) with small, mound-like lobes widely separated, each lobe with about six to eight fine setae inserted towards the base.

LARVA. *Head* (Fig. 12): Antenna shorter than length of head, cylindrical, spined to just beyond insertion of the tuft, which is inserted one-fifth of the distance from the tip; the part beyond the tuft slightly more slender; two long subterminal and one long and one short terminal setae, papilla short and stout. Preclypeal spines stout. Frontal hairs 4, 5, 6, and 7 inserted posterior to the base of the antenna; hair 4 very small, single or with two or three branches; hair 5 large, with three to five branches; hair 6 very long and single; hair 7 shorter, with six to nine branches. Occipital hair 8 double, somewhat longer than hair 9, which has five or six branches. Subantennal hair 12 short, three-branched; ocular hair 14 single or double. Mentum (Fig. 13) with a strong shouldered central tooth and about nine stout teeth on each side. *Thorax:* Skin glabrous. Prothoracic pleural group with one hair long, single; two short, fine, single; and one minute. Mesothoracic pleural group with one hair long, multiple; two long, single; and one minute. Metathoracic pleural group with one hair long, multiple, one long, single, one short, single, and one minute. *Abdomen* (Fig. 14): Long lateral hair 6 of segment I triple, double or triple on segment II; smaller and double or triple on segments III–VI; segment VII with a pair of very long, single hairs. Comb with about 50 scales in a triangular patch; each scale expanded and fringed at the tip. Siphon darkly pigmented, very long and slender, over 10 times longer than the width at base; narrowed beyond the base, then cylindrical to the tip; pecten of 10 or more long, slender, unserrated, sharply-pointed spines, the distal ones longer; four pairs of fine, single or rarely double ventral hairs beyond the pecten, those near the base longer than the apical ones; two pairs of minute dorsal hairs near the apex; terminal hook bifid. Anal segment ringed by the darkly pigmented plate. Inner and outer caudal hairs long, single; lateral hair small, three- to five-branched. Gills variable, the upper pair up to twice as long as the segment; the lower pair about equal to the segment.

TYPES. *Holotype:* Male, with associated larval and pupal skins and mounted genitalia; Acacias, Colombia, 3 June, 1947 (L. E. Rozeboom); from uncut bamboo internodes perforated by holes bored by insects; deposited in the U. S. National Museum. *Paratypes:* Five males and two females with associated larval and pupal skins; a male, a female, and a larval skin, all unassociated. Collected from bamboo internodes at Acacias, Colombia, 3 June, 1947 (L. E. Rozeboom) and at Guamal, Colombia, 13 June, 1947 (Marston Bates). Paratypes deposited in the U. S. National Museum, the British Museum, and the Johns Hopkins School of Hygiene and Public Health.

Taxonomic Discussion.—There are, including *bamborum,* six species of *Isostomyia* Coquillett, characterized by the very short palpi in the male, and by the presence of both outer and inner divisions of the apical lobe on the sidepiece. The males may be separated from one another by the following key:

1. A large, densely setose area on inner surface of sidepiece below the inner division of the lobe 2
   Sidepiece without this setose area 5
2. Inner division of lobe densely setose on under surface half way to tip in addition to setose area on sidepiece ..... *C. canaanensis* L. & W.
   Inner division of lobe not setose on under surface 3

PLATE II

FIG. 8. *C. bamborum.* Sidepiece of male genitalia.
FIG. 9. " " Inner plate of mesosome.
FIG. 10. " " " " " " "
FIG. 11. " " Ninth tergite of adult male.
FIG. 12. " " Head of larva.
FIG. 13. " " Mentum of larva.

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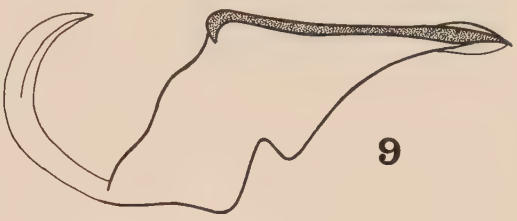

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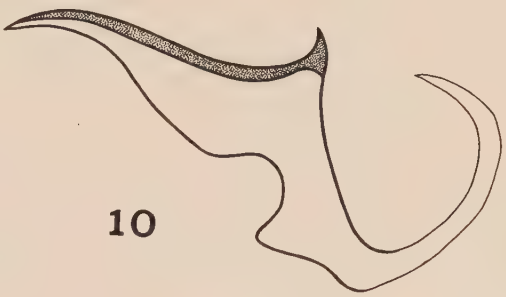

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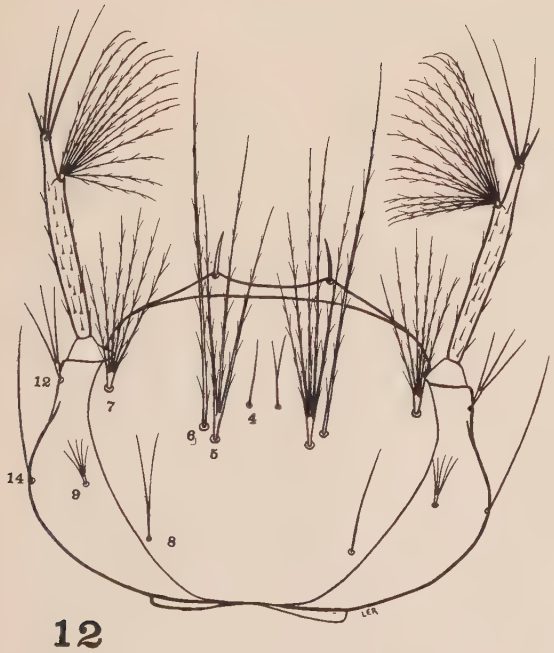

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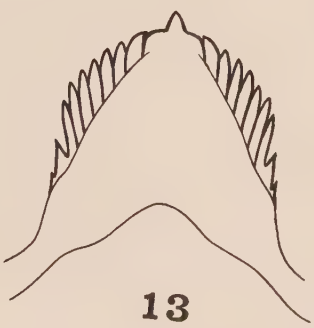

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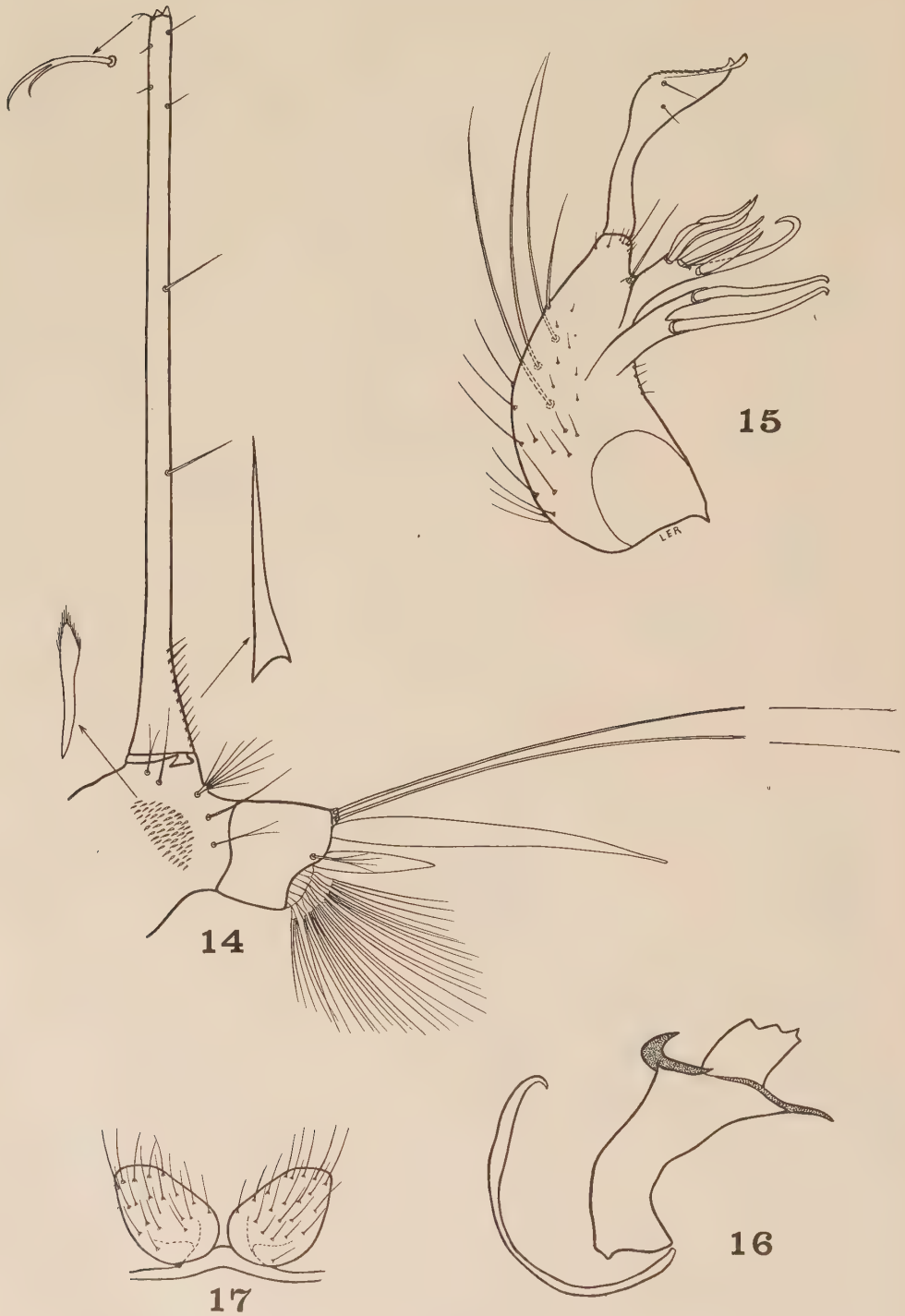


PLATE III

FIG. 14. *C. bamborum.* Terminal abdominal segments of larva.
FIG. 15. *C. batesi.* Sidepiece of male genitalia.
FIG. 16. " " Inner plate of mesosome.
FIG. 17. " " Ninth tergite of adult male.

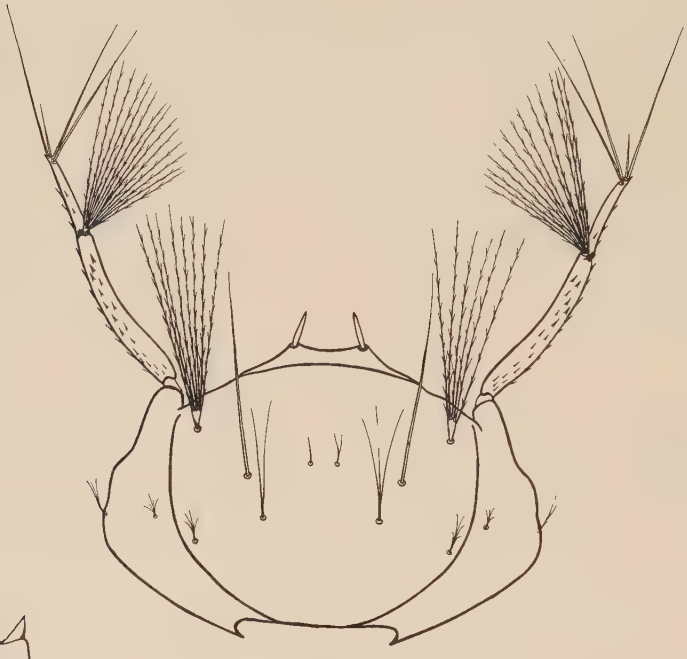
3. Setose area in the form of a swelling or boss; clasper arcuate; outer division of lobe with only one leaf on the stem 4
   Setose area flattened; clasper sharply angled at middle, outer division of lobe with two leaflets near the middle of the stem ..... *C. conservator* D. & K.
4. Outer division of lobe with a seta midway on the stem; inner division shorter, outer arm humped near apex; boss without special enlarged setae ..... *C. browni* Komp.
   Outer division of lobe with an enlarged leaf as well as several broad filaments midway on the stem; inner division long, columnar; distal area of the boss with several enlarged setae among the fine ones ..... *C. bamborum* n. sp.
5. Outer division a small lobe with a few short setae; tenth sternites without subapical setae. *C. corrigani* D. & K.
   Outer division large, with several broad filaments; tenth sternites with a subapical setulose area ..... *C. originator* Gordon and Evans

Culex (*Melanoconion*) *batesi* new species

Female. A small mosquito with dark legs and wings, characterized by the presence of a group of small setae on the postnotum. *Head:* Proboscis dark; palpus dark, less than one-sixth the length of the proboscis; antenna with torus and first flagellar segment brown, the rest of the flagellum darker, with black whorl hairs. Occiput with a dense clothing of broad, flat, black recumbent scales; forked scales dark; scales along eye margins dark; a small patch of white scales laterally. *Thorax:* Prothoracic lobes dark brown, with an uneven row of black bristles. Posterior pronotum with small, curved, dark brown scales along upper margin and a few black bristles posteriorly. Scutum and scutellum clothed with narrow, curved, dark brown scales; bristles black; antescutellar bare spaces pale, greenish brown. Postnotum pale brown, a group about 10 or less small dark setae before the distal margin. Pleurae pale green or greenish-brown, a darker area behind the anterior spiracle and in the mesepimeron; some pale scales and a row of pale setae along posterior margin of sternopleuron; one lower mesepimeral bristle. Fore coxae with dark scales on antero-lateral surface; pale scales on mid and hind coxae. Hind femur somewhat paler below; all other leg segments dark. Scales of wing dark. *Abdomen:* Tergites dark except for very small baso-lateral pale spots on distal segments; sternites greenish, with flat, transparent scales.

Male. Coloration similar to that of the female. Occiput with pale flat recumbent scales intermixed with the darker ones. Palpus exceeding the proboscis by a little more than the length of the terminal segment. *Genitalia* (Figs. 15, 16, 17): Sidepiece with outer margin rounded, inner margin concave; inner surface with short setae; several long strong setae on outer surface. Apical lobe divided; outer division with the outer arm represented only by a prominence in which are inserted four closely appressed, broad filaments; inner arm long, terminating in a long, hooked rod and a shorter, lanceolate filament; a broad, flattened filament and a slender seta inserted between the outer angle and the inner arm. A slender seta inserted in a prominent tubercle on the dorsal surface of the sidepiece near the base of the outer division. Inner division of lobe long, columnar, divided into a longer outer and a short ventral arm; each arm bearing a long stout rod with a hooked tip. Clasper a little over half the length of the sidepiece; the distal third enlarged and narrowing abruptly to the tip, terminating in a blunt, upturned process and a clubbed, appendiculate spine; upper margin of distal third with many small closely appressed lamellae; two fine setae in the enlarged area. Inner plate of mesosome with a broad, somewhat curved stem; a flat erect arm with the upper margin broadly serrate; a long, strong, subterminal dorsal arm; ventral arm subterminal, in the shape of a short, stout hook; basal hook long, curved. Tenth sternite with a comb-like row of short, stout teeth. Lobes of ninth tergite (Fig. 17) large, ovoid, dorsal surface with many slender setae inserted in prominent tubercles.

Larva. *Head* (Fig. 18): Head broader than long; antenna a little shorter than the length of the head, curved, a multiple-haired tuft inserted one-third the distance from the tip, the shaft beyond the tuft more slender; two long subterminal and one long and one short terminal spines; papilla short; outer and dorsal surface of shaft armed from the base to beyond the tuft with stout spines; a broad lamellate, spiny process before the tubercle of the antennal tuft. Preclypeal spines short, stout, darkly pigmented. Frontal hairs 4, 5, 6, and 7 inserted posterior to base of antenna; hair 4 small, single or double; hair 5 double or triple, extending to about the base of the antenna; hair 6 single, long, reaching well beyond the anterior margin of the head; hair 7 large, multiple. Occipital hair 8 small, three- to five-branched; hair 9 small, four- to seven-branched. Ocular hair 14 small, three- to five-branched. Mentum (Fig. 19) triangular, with a large shouldered central tooth and three large, broad teeth on each side. *Thorax:* Skin pilose. Prothoracic pleural group with one hair long single, one short single, one short double, and one minute, three-branched. Mesothoracic pleural group with one hair long multiple, two long single, and



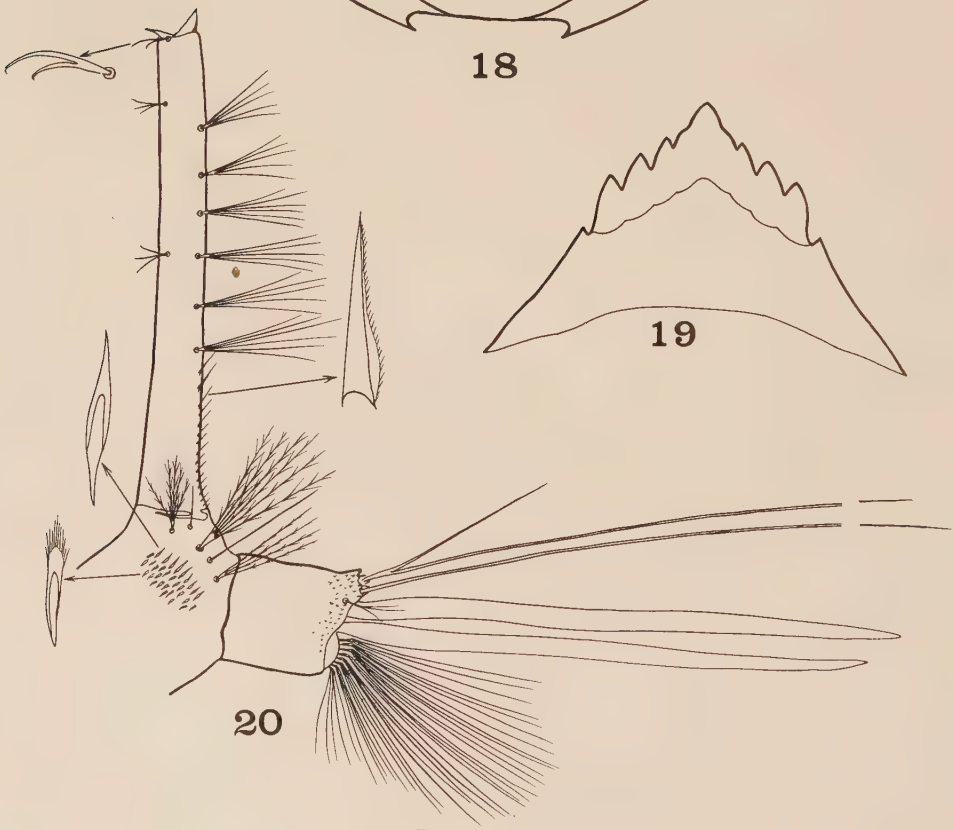


PLATE IV

FIG. 18. *C. batesi.* Head of larva.
FIG. 19. " " Mentum of larva.
FIG. 20. " " Terminal abdominal segments of larva.

one minute. Metathoracic group with one hair long multiple, one long single, one short single, and one minute. *Abdomen* (Fig. 20) : Skin glabrous on proximal segments, finely pilose on distal segments. Long lateral hair 6 double on segments I and II, shorter and three- or four-branched on segments III–VI. Comb of about 30 scales in a patch, those forming the distal row larger; individual small scale of proximal rows fringed at tip; the larger scale of the distal row terminating in a long, pointed spine. Air tube about eight times as long as the width at base, cylindrical, slightly recurved; pecten on basal third, consisting of about 15 teeth, each tooth long, pointed, slender, with a transparent posterior expansion fringed with delicate spines. Six pairs of large, multiple ventral hair tufts beyond the pecten, the proximal tufts a little larger than the distal ones. Two pairs of small, two- or three-haired dorsal tufts. Terminal hook bifid. Anal segment ringed by the plate, the plate spiculate along posterior margin; inner submedian caudal hair double, with one very long and one very short branch; outer submedian caudal hair long, single; lateral hair small, with three or more branches; anal gills very long, about four times the length of the segment, cylindrical.

TYPES. *Holotype:* Male, with associated larval and pupal skins and mounted genitalia; Villavicencio, Colombia, 15 May, 1947 (L. E. Rozeboom) ; collected from partly shaded, shallow margin of stream; deposited in the U. S. National Museum. *Paratypes:* six males with associated larval and pupal skins, four with mounted genitalia; four females with associated larval and pupal skins; collected at Villavicencio and San Martin, Colombia (L. E. Rozeboom) from streams, pools, and swamps. Paratypes deposited in the U. S. National Museum, the British Museum, and the Johns Hopkins School of Hygiene and Public Health.

Taxonomic Discussion.—The most distinguishing feature of *C. batesi* is the postnotal tuft of setae in the adult, a character which has been described for no other species of *Melanoconion.* However, it is doubtful that many *Culex* species have been examined carefully for these setae. The inner plate of the mesosome of the male, with a serrated upper arm and two subterminal arms, is similar to that of several other *Melanoconion.* These species, and the characters for differentiation between them and *batesi,* are as follows:

C. albinensis B.-W. and B. According to Bonne and Bonne-Wepster (1925) the postnotum is nude, while the shape of the inner plate of the mesosome and the appendages of the outer division of the lobe are unlike these structures in *batesi.*

C. amitis Komp. Komp's (1936) description shows the species to differ in the shape and serration of the upper arm of the mesosome and in the lobes of the ninth tergite.

C. bonneti Senevet. This species appears to be very close to *batesi.* From descriptions by Senevet (1938) and Floch and Abonnenc (1945), the most obvious differences in the male of *bonneti* are the presence of an expanded leaf instead of a broad filament between the outer angle and inner arm of the outer division of the lobe, the absence of the seta inserted at the same level with this leaf, and the shape of the inner plate of the mesosome, which is divided into two arms apically and lacks the subterminal hook. The larva of *bonneti* is described as having five pairs of ventral siphon tufts instead of six, and frontal hair 5 is single instead of double or triple.

C. corentynensis Dyar. This species is said by Dyar (1928) to possess a narrow leaf on the outer division of the lobe, and no mention is made of the subapical and basal setae which are so conspicuous in *batesi.*

C. elephas Komp (1936) is readily distinguishable from *batesi* in the structure of the clasper, the apical lobe, and ninth tergite.

C. evansi Root (1927) bears an expanded leaf on the lobe of the sidepiece.

C. flabellifer Komp (1936) has an expanded leaf above the lobe, while the shape and denticulation of the upper arm of the mesosome are unlike those of *batesi.*

C. limacifer Komp (1936) has the upper arm denticulate along the dorsal margin instead of at the top, and the lobes of the ninth tergites are produced upwardly at the inner angle.

C. lucifugus Komp (1936) is separable from *batesi* especially in the shape of the mesosome.

C. madininensis Senevet (1936) bears a leaf on the outer division of the lobe, and besides this, only two broad filaments. The mesosome and clasper are unlike those of *batesi.*

C. maroniensis B.-W. and B. According to Bonne and Bonne-Wepster (1925) the postnotum is nude, while the upper arm of the mesosome is expanded and serrated with many small teeth.

C. phlogistus Dyar. The upper arm of the mesosome is shown by Dyar (1928) to be rather tall and broad, with many fine serrations.

C. plectoporpe Root (1927) possesses a leaf on the outer division of the lobe, while the upper arm of the mesosome is finely serrated.

C. serratimarge Root (1927) is readily distinguished by the large ninth tergite lobes.

C. batesi is named in honor of Dr. Marston Bates, Director of the Rockefeller Foundation Laboratory at Villavicencio, Colombia.

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ADDITIONAL OBSERVATIONS ON THE BIONOMICS AND LIFE HISTORY OF *GORGODERA AMPLICAVA* LOOSS, 1899 (TREMATODA: GORGODERIDAE)

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INTRODUCTION

Life history studies have been made on several species in the trematode subfamily GORGODERINAE with varying success; at present, the life cycles of three American species are known and there is rather good evidence on the life cycles of a few other American species and several European ones. In every case so far reported, the first intermediate host is a bivalve mollusc and the definitive host is an anamniotic vertebrate.

The description of the life cycle of the bladder fluke, *Gorgodera amplicava,* as reported by Krull (1933, 1935), is correct, but additional facts of general interest have been discovered in experiments conducted during the summers of 1943 through 1947 on Cape Cod, Massachusetts. Some of these findings have already been published in an abstract, Goodchild (1945) : the present paper extends the information on the bionomics and life history of *G. amplicava.*

Literature on life cycles of gorgoderid trematodes was reviewed by Rankin (1939) and Goodchild (1943). Sinitsîn (1905) was the first investigator to attempt experimentally the linking of an adult bladder fluke with the characteristic large-tailed gorgoderid cercaria. Krull reported that cercariae of *G. amplicava* were produced in the sphaeriid mollusc, *Musculium partumeium* (Say), that they encysted as metacercariae in the snail, *Heliosoma antrosa* (Conrad), and that metacercariae in infected snails when eaten by *Rana clamitans* Latreille or *R. catesbeiana* Shaw grew eventually into *G. amplicava* adults. Rankin (1939) traced the life cycle of *Gorgoderina attenuata* Stafford which utilized *Sphaerium occidentale* Prime as the first intermediate host, tadpoles of several species of *Rana,* larvae of *Triturus* and the snail, *Pseudosuccinea columella* (Say), as metacercarial transfer hosts, and various species of *Rana* and *Triturus v. viridescens* Raf. as final hosts. Goodchild (1940, 1943) determined the life history of *Phyllodistomum solidum* Rankin from the urinary bladder of the plethodontid salamander, *Desmognathus fuscus fuscus* (Raf.). In this cycle, *Pisidium abditum* Haldeman served as the first intermediate host and the active cercariae were eaten by odonatan larvae in which they became metacercariae.

MATERIALS AND METHODS

The animals used in this study were collected from many small ponds near Woods Hole, Massachusetts, and in southwestern Missouri. The small bivalve molluscs, *Musculium partumeium,* used in the miracidial infection experiments, were collected in the field and successive generations, over a period of four years, were reared in laboratory cultures as a source of uninfected material. Naturally and experimentally infected bivalves were used as a source of mother and daughter sporo-

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cysts for studying the motion, the rate of development and other aspects of larval behavior. Tadpoles, often 100 per cent infected and with as many as 300 metacercariae in one host, were taken in large numbers from the same ponds as the infected bivalves. Naturally infected bullfrogs were collected from the same habitats as the molluscs and tadpoles; these frogs, as well as experimentally infected ones, were used as a source of adults which produced miracidia for first intermediate host experiments. Parasite-free *Rana catesbeiana,* tadpoles and adults, collected along the James River and its tributaries in southwestern Missouri, were used as controls in feeding experiments. Moreover, all stages in the life cycle have been followed at such close intervals that a continuous series of developmental stages has been obtained.

Infected laboratory-cultured clams and field-collected ones were used as a source of cercariae. The cercariae, which were given off usually late in the day, were studied that evening. Very dilute (stock solution 1:1000 in distilled water) neutral red was used to determine the number of penetration glands and the staining reaction of their granules. No suitable histological technique has yet been found to prevent distortion and collapse of the cercarial tail in the preparation of permanent slide mounts; however, ten per cent formalin is suitable to kill and preserve these cercariae without distortion.

Metacercariae have been recovered from naturally infected tadpoles and the snails, *Pseudosuccinea columella* and *Physa heterostropha* (Say). Tadpoles were fed on recently emerged cercariae, often under observation with the binocular dissecting microscope. These hosts were sacrificed after increasing intervals of time to trace the path of penetration and the sites and methods of encystment. Single hosts were fed cercariae over a period of time and fixed while still engulfing cercariae to obtain all stages of metacercarial development in the same host. Moreover, snails, larval salamanders, *Ambystoma maculatum* (Shaw), and odonatan larvae have been fed cercariae of *G. amplicava* successfully in the laboratory; the results of these feeding experiments are discussed in the section dealing with experiments.

Adult worms recovered from the bladder have been studied alive and in permanent preparations. Young adult worms have been taken from the bladder, and in addition, have been found in the stomach, intestine, cloaca, Wolffian ducts and oviducts. Bouin's, Gilson's, Carnoy's (6:3:1), medium Flemmings's and ten per cent formalin have been used as fixatives. Paracarmine, borax-carmine, and Heidenhain's hematoxylin have been used as stains, and specimens were prepared both as whole mounts and as sections.

The miracidial epidermal plates were demonstrated by a modified silver-line technique. Free-swimming miracidia were pipetted in minimum quantities of water into cold one per cent silver nitrate solution and allowed to remain there for from ten to fifteen minutes. They were then washed very quickly through two changes of distilled water and exposed to brilliant sunlight for one hour. An alternative method which allowed the miracidia to remain in the silver nitrate solution for several hours in subdued light gave equivalent results. Water mounts with No. 1 cover glasses were made and glycerine was allowed to diffuse slowly into the preparations over a period of several days. By manipulating the edge of the cover glass with needles the miracidium could be rotated into all positions including anterior and posterior vertical views, Figs. 3 and 4. The edge of the cover glass was after-

wards sealed with asphaltum or Fisher's Sealit to make a semi-permanent preparation.

The author wishes to thank Dr. Paul Bartsch of the United States National Museum for the identification of the molluscs used in these experiments.

EXPERIMENTS

1. *Experiments with first intermediate hosts:*

Infected bullfrogs were induced to void urine containing the ripe eggs of the trematode. The frogs were grasped gently around the head with the thumb and index finger encircling the body behind the forelegs. After the hand was in correct position the grip was suddenly tightened. The frog reacted instantly and in its struggling motions to escape, invariably discharged quantities of urine into a shallow Stender dish held over the vent. The urine was then diluted with tap or pond water and shortly (almost immediately to a day afterward) the miracidia were liberated from the eggs. These first stage larvae were directed with micropipettes into the extended incurrent siphons of actively feeding molluscs and were drawn into the mollusc through the siphon or ventral mantle cleft. The larvae were not attracted to the molluscs and no active penetration from the outside was ever observed. In one case, a miracidium, caught in a mild incurrent flow, was seen to brush against the lip of the incurrent siphon; it became fixed to the siphon where it remained for approximately 30 seconds undergoing slight body movements; finally, it became detached and swam away.

The miracidia were, in all probability, drawn up against the gill filaments by the straining action of the mollusc's cilia and from there effected their entrance into the gill substance. This action was not observable through the opaque shell; however, by placing swimming miracidia on living gill fragments the methods of attachment and penetration were readily determined. The anterior end of the larva adhered to the gill tissue and the body became oriented perpendicularly to the gill. Penetration required approximately ten minutes and was accomplished by rythmical anteroposteriad waves of contraction which resulted in gradual disappearance of the miracidium into the gill tissue. Since the miracidium is devoid of boring spines, stylets, or other sclerous penetrating aids, the successful invasion of the mollusc must depend on these muscular contortions with perhaps localized gill histolysis caused by miracidial glandular secretions.

2. *Experiments with second intermediate hosts:*

Bivalves which were actively shedding cercariae were placed in individual containers. This isolation served several purposes: it enabled the animals to live for a longer time in the laboratory because special care could be given to the infected ones, and, it allowed easier and quicker recovery of the cercariae because they could be confined in a smaller space and were not tangled by the crawling of other molluscs. Approximately thirty to fifty cercariae were liberated, during the late afternoon and early evening, from the excurrent siphon of the host animal, Fig. 42. These recently emergent cercariae were used in feeding experiments with the following intermediate hosts. Typical experiments will be described:

a. *Tadpoles*—Tadpoles of several anuran species including: *Rana catesbeiana, R. clamitans, R. palustris* LeConte and *Hyla versicolor* LeConte ate the large-tailed cercariae. Rankin (1939) pointed out that larval anurans are, "bottom feeders, sub-

sisting on algae, organic debris, etc." The cercariae of *G. amplicava* were taken in with the food and, in addition, the respiratory currents of the tadpole were sufficient to suck in the long, transparent, but tough tails of the cercariae through the mouth (*Rana*) and external nares (*Hyla*). Once the tail was engulfed, the remainder of the cercaria, including the anterior chamber containing the young distome, was drawn into the mouth of the tadpole. The following unusual reaction of some *Rana* tadpoles has been observed on several occasions: when the tail of a cercaria had been drawn into the mouth, accelerated oral motions of the tadpole caused the tail to be taken in more rapidly; however, the horny jaws suddenly severed the tail just behind the anterior chamber and the mouth, by a violent reversal of motions, forced the still enclosed young distome away from the tadpole.

Young distomes emerged from the anterior chambers of the cercariae in the mouth cavity, esophagus, stomach and intestine of the tadpole. They have been recovered, while still crawling in their leech-like manner, from the inner surfaces of these organs and have been taken while penetrating the wall. Penetration of the gut wall required approximately 10 to 15 minutes, and there were no violent reactions to the process of cercarial penetration as were described in the odonatan nymphs by Goodchild (1943). In practically every case, the cercaria penetrated merely the gut wall; it did not continue on through the peritoneum. The huge abdominal masses of metacercariae were covered over with thin vascularized mesenteric or visceral peritonea.

The cercaria started to encyst soon after penetration. In one experiment, a tadpole was sacrificed twenty minutes after being fed one cercaria. The young metacercaria in forming its protective cyst adjacent to the stomach, threw its body rather slowly into U-shaped and loose S-shaped figures which afforded an opportunity for the anterior part of the worm to distribute and smooth uniformly over the inner surface of the forming cyst the cystogenous material emitted from the posterior opening. Because the body wall of the tadpole was opaque, these processes could not be seen *in situ;* and moreover, it was impossible to keep any one metacercaria under observation over a period of time and thereby to determine its rate of growth, to ascertain the possible augmentation of the metacercarial cyst by the action of the host, to study the internal changes taking place within one particular worm, or to note the motion of the animal within its cyst. The metacercaria was infective for the final host after approximately 24 hours in the tadpole.

b. *Urodele larvae*—Small larval salamanders, *Ambystoma maculatum,* have been successfully fed these large-tailed cercariae. Because salamander larvae are predaceous and will voluntarily take as food only moving objects, the motionless cercariae were jiggled with glass capillaries to induce the amphibian larvae to devour them. Forced feeding also was employed.

In one experiment, a small *Ambystoma* larva was fed six cercariae; after ten minutes the mouth and stomach were opened and examined. One of the cercariae had penetrated and was found encysting subperitoneally along the dorsal side of the coelom. It was studied as it prepared its cyst wall and until it died, through exposure, twenty minutes later. Young distomes have also been recovered from the submucosa of the mouth cavity after whole cercariae had been eaten. Dead and partially digested distomes surrounded by matted and amorphous tails have been observed in the stomach cavity. Apparently *Ambystoma* larvae can serve as meta-

cercarial hosts if the young distomes emerge from the anterior chamber quickly; their importance as natural vectors would be negligible because normally the urodeles would not feed on motionless cercariae.

c. *Snails*—Large gastropod molluscs, *Pseudosuccinea columella* and *Physa heterostropha,* were found infected naturally with metacercariae of *Gorgodera amplicava.* In some small ponds nearly every one of these snails harbored from one to eight or more metacercariae. Experimental metacercarial production in these molluscs was obtained also. In one case, a specimen of *P. columella* was sacrificed soon after it had been fed three cercariae. In the peri-esophageal hemocoel, four worms were discovered; one of these was an old, encysted metacercaria with well-defined excretory concretions in the bladder; one was barely starting the formation of a cyst wall which was still exceedingly thin and fragile; the two remaining unencysted distomes were crawling about in the hemocoel.

Krull (1936) determined that *Physa halei, Lymnaea traskii, Heliosoma trivolvis* and *Pseudosuccinea columella,* in addition to *Heliosoma antrosa* would serve as metacercarial hosts. He stated further: "Since snails from several widely separated genera have been shown to be experimental second intermediate hosts, it may be assumed that the majority of pond snails will serve in this capacity."

d. *Odonatan larvae*—Odonatan nymphs occurred in the same ponds as infected bivalves, but, as yet, no damselfly or dragonfly larva naturally infected with the metacercariae of *G. amplicava* has been discovered. The predaceous nature of these larvae also prevented their becoming infected; however, the jiggling technique used to induce the urodeles to feed on the cercariae was successfully used with odonatan larvae. In a typical case, a rather large *Enallagma sp.* was fed a group of tangle cercariae. One-half hour later the insect was opened and examined. Three unencysted distomes were recovered from the thoracic hemocoel. Eight cercariae were observed creeping about on the inner surface of the crop, and two of these actively penetrated the intima and began to encyst between the inner and outer crop wall.

Transplantation of metacercariae from the tadpole host to the dragonfly was attempted, but the wounding of the dragonfly nymph to allow introduction of the metacercariae quickly killed it.

Martin (1937) reported another fresh-water arthropod as a second intermediate host. He found over fifty per cent of the crayfish, *Cambarus sp.,* from a small stream near Lafayette, Indiana, to be infected with these metacercariae.

e. *In vitro*—If young distomes were removed from the tadpole host immediately after they had penetrated the gut wall and before they had started to encyst and were placed in plasma of the tadpole, they began the typical encysting contortions, but were incapable of forming a cyst wall. The impression obtained, after comparing the normal and the *in vitro* process, is that the animal needed external support for the formation of its cyst wall. For this reason, a different experiment was devised to provide this support. Cercariae were placed upon the opened and stretched stomach and duodenum of a tadpole pinned to the surface of two per cent agar-agar made up in amphibian Ringer's; they emerged, at once, from the anterior chamber and penetrated the gut wall within thirty minutes. Typical encysting motions were then started in the agar-agar; however, the majority of the cercariae did not complete their cysts and feeding experiments were not conducted with those that did complete them. More experiments of a similar type are planned; with proper experimental technique it is believed that the metacercarial host can be eliminated.

f. *Metacercarial ectopia*—The distomes in cercariae left in pond water remained in the anterior chamber until dissolution of the whole larva was complete. However, if a cercaria was placed in weak hydrochloric acid solution, the distome readily emerged. At pH 1 the enclosed distome became active in 5–10 seconds and immediately emerged from the anterior chamber which quickly became amorphous and gelatinous. The emergent distome was not normal; it was capable of spasmodic movements only and after 2–3 minutes rounded up and became insensitive to mechanical stimulation. At pH 1.5 activation occurred in 5–10 seconds and emergence commenced after 15–60 seconds. The distome stopped at the acetabular level and waved randomly the extended anterior end in supposed grasping attempts. If the oral sucker touched the substrate or other solid object it anchored and pulled the remainder of the body from the anterior chamber. This action could serve the purpose, in the intermediate host, of locating and attaching the oral sucker to the gut mucosa prior to penetration. Emergence occurred within 2–4 minutes even in the absence of solid support anchorage. Cercariae could not tolerate outside existence at this pH and within 5–8 minutes the animal succumbed and assumed an elongated and swollen shape. Slightly delayed preliminary excitement was noted at pH 2 and pH 3, and emergence ceased when the ventral sucker reached the apical chamber opening. At pH 4 the recurved anterior end of the distome straightened after about 15 seconds but quickly resumed the typical recurved position and thereafter, the animal remained quiet. At pH 5 and above no visible cercarial reaction was noted.

Using these pH data, metacercarial ectopia in tadpoles was attempted. Cercariae were placed on pieces of living tadpole tail in pH 1.5 hydrochloric acid. The young distome, after emergence, crawled about on the outer surface of the tail and in from 1–5 minutes had attached firmly by the ventral sucker and had started to attack the tail epithelium with the stylet (Fig. 44). Several larvae successfully penetrated at the first attempt and in about 4 minutes had started typical encysting motions; the animal, after an initially vigorous ten minute cyst-forming period, gradually slowed down becoming relatively inactive in 30–45 minutes. Other larvae did not successfully invade at the first penetration attempt. These worms scratched an epithelial hole into which the oral sucker and prosoma penetrated (Fig. 45); however, the probing of the anterior end forced a second hole through the epithelium (Fig. 46) and, describing a U-shaped turn, the opisthosoma entered the first hole (Fig. 47) and the whole worm re-emerged through the second hole back to the surface (Fig. 48). Second and third penetrating attacks were made and in most cases the distome ultimately successfully invaded and encysted.

3. *Experiments with final vertebrate hosts:*

The behavior of metacercariae in the several amphibian final hosts was strikingly uniform. Excystment of the worms could take place in the stomach of the host, but the small intestine was the more usual site. When finally liberated from the cysts, the young bladder flukes migrated posteriorly by crawling along the mucosa of the small and large intestine. In the cloaca the worms sought out and entered the openings of the excretory or reproductive ducts. Even in normal hosts, however, the migration of the worms was not without peril. Some cysts were held in the stomach in large masses of digesting flesh until the imprisoned worms died, while some worms became trapped in fecal pellets and were unable to extricate

themselves before being eliminated. Moreover, young distomes were eliminated from the bladder or cloaca in forceful urination.

An experiment with *Rana palustris* performed with metacercariae of *G. amplicava* is typical of many others performed with this and other frog hosts: the frog was fed one infected tadpole containing approximately 30 metacercariae at 12:00 noon; at 2:00 PM a second tadpole with about the same number of cysts was given to it; finally at 5:00 PM a third tadpole with 20 metacercariae was forced into the stomach of the frog. At 8:00 PM of the same day, the frog was killed and examined. The stomach held a mass of digesting flesh enclosing many encysted, but active, gorgoderid trematodes. The first excysted worm was located 24 mm beyond the pyloric sphincter (the total length of the small intestine was 76 mm). From this point to the junction of the small and large intestine more excysted than encysted metacercariae were present; in the posterior portions of the ileum excysted worms only were found. In the large intestine the excretory bladder concretions of the worms were discharged. Immature worms of the same size and characteristics were recovered from the seminal vesicles and more distal portions of the Wolffian ducts. The urinary bladder of this particular frog was negative for gorgoderid trematodes. In other cases, however, young distomes have migrated into the bladder of the frog directly from the cloaca. In this host, migration of the worm through the gut and invasion of the execretory system was accomplished in eight hours or less from the time of the initial metacercarial feeding.

Rana catesbeiana, R. clamitans, R. palustris, R. pipiens brachycephala (Schreber), *R. pipiens sphenocephala* Cope, and *Bufo americanus* Holbrook were all suitable final hosts for laboratory-fed metacercariae of *G. amplicava.* The young distomes excysted and were able to establish themselves in the urogenital ducts of all the anuran hosts tested.

Urodeles have been tried as final hosts. One 2-inch long female *Ambystoma maculatum* was fed a tadpole containing approximately twenty encysted metacercariae. The next day the animal was sacrificed and examined. The urinary bladder, exposed first, was free of distomes. The digestive system opened posteriorly uncovered a fecal mass containing 10 living, but still encysted, metacercariae and three excysted normal-appearing, actively-crawling young worms. No excysted or encysted metacercariae were present in either the small intestine or stomach. The mesonephroi and their ducts were also devoid of young worms. Four young excysted worms in contiguous tandem were discovered, two-thirds of the way up the right oviduct. One worm, at the same level, was found in the left oviduct. This case is not unique because excysted metacercariae have been recovered also from the oviducts of *R. pipiens brachycephala.*

Normal excystment of the metacercariae of *G. amplicava* occurred in the small intestine of the newt, *Triturus v. viridescens,* but they were unable to invade the excretory or reproductive ducts. Young distomes were able to attach and to crawl on the mucosa of the ileum, but in the large intestine they were apparently forced away from the wall by the activity of mucous glands whose secretion further trapped them in slimy fecal pellets until they were eliminated. In only one case was a worm found attached to the wall of the large intestine. The failure of *G. amplicava* to infect this urodele host may, therefore, be due to this mechanical mucous barrier, but the fact that worms may occasionally reach the cloaca and then fail to penetrate the

uro-genital ducts requires additional explanation. Strong cloacal ciliary action, in addition to mucous secretion, could prevent penetration of the cloacal ducts. *Gorgoderina bilobata* Rankin and *G. attenuata*, according to Rankin (1937, 1939), and *G. intermediata* Holl, according to Holl (1928), can successfully invade *T. v. virides-cens*. The success of these worms may be due to their possessing more powerful or efficient suckers allowing normal migration in the amphibian host after ex-cystment.

DESCRIPTIONS OF STAGES IN THE LIFE CYCLE

1. *Miracidium*

The miracidium is here described in detail because Krull (1935) did not mention this stage in his account and because in the literature there is conflict regarding several points of gorgoderid miracidial anatomy.

The non-operculate eggs (Fig. 6), 55 μ by 39 μ, have completed embryogeny by the time they reach the genital pore of the sexually mature worm. They are oviposited into the bladder of the host and hatch explosively soon after the urine in which they are suspended is diluted with tap or pond water. The free miracidium (Fig. 7), approximately 55 μ long by 28 μ wide, is a deliberate swimmer; it moves forward rotating slowly on its long axis with a slight wobbling motion. If it brushes against an object in the water it stops, turns in a new direction and proceeds forward again. The larva can swim for approximately 48–72 hours at room temperature (middle 70°); if it has not been drawn into the next host within that period of time it slows down gradually, finally falling to the bottom and disintegrating.

The free-swimming, newly-hatched larva is pyriform (Fig. 8), but it is capable of a limited metaboly (Figs. 9, 10, 11, 12); during the second day the pyriform shape is reversed and the small end is forward (Fig. 13). The outer surface of the body is covered with vibratile cilia, approximately 5 μ long, arising from conspicuous parallel rows of basal granules located about 2–3 μ apart in the epidermal plates. Cilia start beating in the unhatched miracidium and their posterior thrust is easily studied there by watching the movements of small inclusions floating between the miracidium and the very thin shell wall. Cilia are absent from a small, 3–4 μ in diameter, hexagonal area at the anterior tip (Fig. 3) and from the interepidermal plate areas, although these latter areas are not readily apparent *in vivo* because of overhanging cilia. Many of these structures are most graphically delineated when the miracidium is well-flattened and about to burst; at that time, the cilia-free interplate areas are clearly shown and the extreme thinness (1 μ) of the epidermal plates is visible as they pull away from the subepithelium.

The fifteen epidermal-plate cells, counted in living larvae and in the silver nitrate killed ones, are arranged in three interlocked tiers (Figs. 1, 2, 3, 4). The

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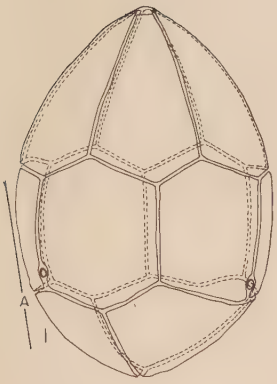
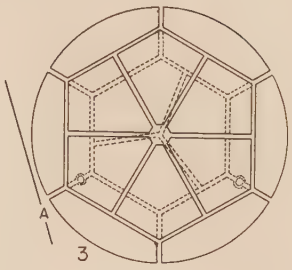
EXPLANATION OF PLATE 1

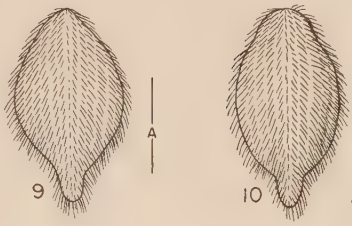
All figures, except as noted, were made with the aid of a camera lucida to the following scales: A scale = 25 μ; B scale = 50 μ; C scale = 0.25 mm; D scale = 0.50 mm; E scale = 1.0 mm.

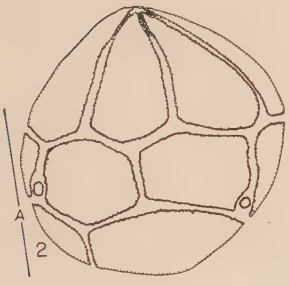
FIG. 1. Miracidium, ventral view of epidermal plates, diagrammatic.
FIG. 2. Miracidium, ventral view of epidermal plates.
FIG. 3. Miracidium, anterior view of epidermal plates, diagrammatic.
FIG. 4. Miracidium, posterior view of epidermal plates.
FIG. 5. Miracidium, showing silver nitrate stained glands.
FIG. 6. Egg with miracidium (free-hand).
FIG. 7. Miracidium, lateral view (free-hand).
FIGS. 8–13. Free-swimming miracidia.

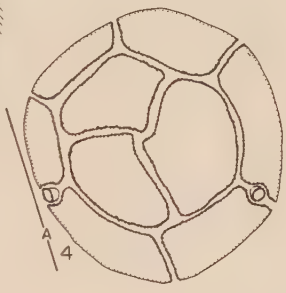
PLATE I

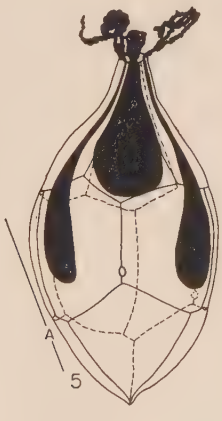


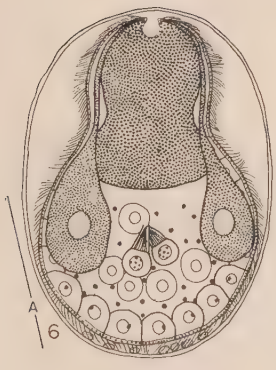


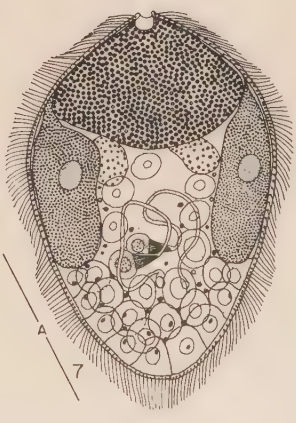












anterior tier is composed of six acutely triangular cells, approximately 25 μ long and 12 μ wide, with slightly truncate anterior apexes, and convexly obtuse bases extending at least a third of the way posteriorly. The middle tier is composed of six roughly rectangular cells, approximately 17 μ long and 20 μ wide, with medianly bulged ends fitting snugly into the undulated posterior margin of the anterior tier. The posterior tier is composed of three nearly equilateral triangular cells, approximately 15 μ long, whose wavy bases are interlocked with the posterior margin of the middle tier. The sum of these lengths is slightly greater than the miracidial length because of overlapping measurements.

The excretory pores (Fig. 4) are 2 μ in diameter and open to the surface from shallow subepithelial vesicles in slightly expanded interepithelial plate areas approximately 4 μ anterior to the junction of the middle and posterior rows of plates. The edges of the pores, the cavities of the subepithelial vesicles and the linings of the two excretory canals become intensely blackened with silver nitrate as used for determining the epithelial plate pattern. The pores are about the only convenient points of topographical reference in this biradially symmetrical animal and merely for the sake of organ position description are here called ventrolateral.

The subepithelium, which becomes the wall of the mother sporocyst (Fig. 6) upon penetration into the gill of the bivalve host, is composed, posteriorly, of well-defined cells, 5–10 μ in diameter. The cytoplasm of these cells is faintly granular and stains a light red with neutral red while the nuclei are large and each contains a large peripheral granule. In the anterior third of the larva, lateral to the medial apical gland cell and the two paired gland cells, the subepithelium is not clearly visible.

A small apical depression, 3 μ in depth and 3 μ in diameter, is visible in the unhatched and often in the free-swimming larva. Surrounding and extending posteriorly from the depression is an anterior, median gland and adjacent is a dorsal and ventral pair of glands which open apically and stretch posteriorly at least half the body length (Figs. 5, 6, 7). Before hatching, the glands are filled with motionless granular material the individual particles of which are about 0.5 μ in diameter. Within a minute or two after hatching, the granules in the median terminal gland enlarge to about twice their former size and start an active Brownian movement probably by the rapid absorption of water by the granules and a hydration of the granular matrix. The granules in the paired glands start Brownian movement, but do not increase in size. The particles of the apical gland remain relatively colorless with dilute neutral red, whereas, the granules of the other two glands stain an intense red color. It is suggested that the median structure is an adhesive gland while the others are penetrating glands. The rapid apical adhesion of the miracidium at the onset of penetration and a staining reaction with neutral red of the paired glands identical to that of the cercarial penetration glands supports this proposal. The apical gland in the miracidium shown in Fig. 7 is 19 μ long by 33 μ wide; the larger of the two slightly dissimilar paired glands is 28 μ long by 8 μ wide, the smaller is 24 μ long and 9 μ wide. This slight inequality is visible only under the higher powers of the microscope. With reflected light using the binocular microscope, the two glands in a swimming larva look identical and their gleaming-white, rotating contents resemble the turning balls on a governor. Clear, vesicular nuclei, 5–7 μ long by 3–5 μ wide, and apically opening, narrow gland ducts are

visible in some favorable unhatched miracidia, especially when the anterior end is narrowed and thrust forward (Fig. 6) and in larvae killed with silver nitrate to determine the epidermal plates (Fig. 5).

Two large laterally-placed flame cells, 8 μ in diameter, with characteristic nuclei 3–5 μ (Fig. 7) are located slightly behind the middle of the body. The vibratile ciliary tuft which is 3 μ wide at the base and 6 μ long is visible in nearly every miracidium including those still unhatched (Fig. 6). Excretory ducts run from the cells anteriorly a short distance, then they bend posteriorly and follow tortuous courses to near the posterior end where they turn anterolaterally to open at the bases of the subepithelial excretory vesicles (Fig. 7).

A few cells with very large nuclei are located in the interior of the miracidium behind the flame cells; these may be the germinal elements of the next generation as has been suggested by Rankin (1939) and Goodchild (1943). However, the cells here are not so definitely isolated as Rankin shows for the miracidium of *Gorgoderina attenuata*. Near the anterior end and partly or completely masked by the glands are several granular units (Fig. 7) of undetermined function. In addition to these internal cells there is a small irregularly-shaped internal cavity, several clear vesicles 6–10 μ in diameter, and many very small, about 2 μ, clear fat-like droplets.

2. *Mother Sporocyst*

During the first hour the miracidium becomes firmly anchored in the gill tissue. The ciliated epithelium begins to lift off the subepithelium (Fig. 14) as a continuous layer, indicating that the larva possesses a strong interepithelial plate cement which binds the outer cells together. The miracidium of *G. attenuata* differs from this one, for Rankin (1939) stated: "The clear, non-ciliated areas between the epidermal plates are the exposed parts of the sub-epithelium over which lie the plates." The subepithelial plate space contains many small particles exhibiting Brownian movement, and the intact investing epithelium has been noticed as long as 43 days after miracidial penetration.

In the very young sporocysts (Figs. 15, 16, 17, 18) miracidial polarity is lost and the subepithelium is very evident. This layer is composed of large cells, from 7–14 μ in diameter, with large vesicular nuclei, approximately 4–5 μ in diameter, containing fine peripheral granules. Moreover, uniformly-sized spherical cells, 8 μ in diameter, with hyaline cytoplasm and highly refractive nuclei, 2 μ in diameter, as well as clear vesicles and many small droplets ranging down to about one micron in diameter, are scattered among the larger outer cells. Several transparent areas located at one edge of the young mother sporocyst (Fig. 14) may represent the empty miracidial gland cells. Three large irregularly shaped cells, 15 μ in diameter, with clear nucei, 8 μ in diameter, are present in the wall of the sporocyst; they are very conspicuous because of their coarse granular cytoplasm. The origin and significance of these cells is, at present, uncertain. They may be persistent miracidial cells, displaced remnants of the terminal gland cells, or special cells which differentiate in the molluscan host. Although definite muscle cells were not observed, the young larva, as well as older ones, retains slight ability to move.

The excretory ducts of the mother sporocyst were not determined because the supporting host tissue decreases visibility and makes controlled flattening difficult. An unusual and gradual metamorphosis of the miracidial-type flame cell of the early

PLATE 2

mother sporocyst produces, after about 40–50 days, the giant semilunar-shaped ones previously known to exist in the daughter sporocysts. This change, indicated in Figs. 21 through 27, is accomplished without any increase in flame cell number. Moreover, giant flame cells are not limited to larval stages because flame cells identical in appearance to Fig. 26 have been discovered in an old adult of *Gorgodera amplicava* (Fig. 74).

The central cavity of the maturing sporocysts becomes increasingly larger and is easily detected in the four-day-old mother sporocyst (Fig. 15). Small germ balls which develop in the wall of the sporocyst are passed into the central cavity where they slowly mature to form the single generation of daughter sporocysts (Fig. 19 and 20).

3. *Daughter Sporocyst*

The description of this stage given by Krull (1935) was confirmed by the present study. However, some sporocysts containing fully-formed cercariae were larger than Krull's specimens; they measured up to 4.1 mm long by 0.39 mm wide.

Huge masses of tubular, unbranched and sluggishly moving sporocysts are located chiefly ventrally between the inner and outer gill lamellae in both pairs of gills. They do not extend into the mantle cavity as Rankin (1939) has reported for sporocysts of *G. attenuata,* but are always surrounded on both sides by gill tissue. The somewhat constricted anterior end is imbedded, apparently as a holdfast organ and perhaps as a food getting organ, in the host gill while the rounded posterior end is free. A birth pore is located terminally on the anterior end (Fig. 28) and complete cercariae pass through it and into the epibranchial chamber from which they are eventually shed to the outside through the excurrent siphon (Fig. 42).

The wall of the sporocyst is about 8–10 μ thick everywhere except at the lips of the anterior opening and at the posterior tip where it is slightly thicker. The wall is roughened externally and is provided with a thin cuticula beneath which are delicate muscle fibers slightly more abundant anteriorly. Giant flame cells (Fig. 26) are identical to those previously described from gorgoderid sporocysts.

4. *Cercaria*

Krull's (1935) account of the infective cercariae is complete and accurate and it will not be duplicated here. However, as pointed out by Goodchild (1945) the tail has been found to be much longer than previously reported; recently shed cercariae range in length from 12–17.4 mm. (average 13.9 mm.).

The intra-sporocyst development of small, active gorgoderid cercariae was described first in detail by Thiry (1859) who studied *Cercaria macrocerca.* Since the

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Explanation of Plate 2

Fig. 14. Mother sporocyst *in situ,* 30 minutes old.
Fig. 15. Mother sporocyst, 4 days old.
Fig. 16. Mother sporocyst *in situ,* 10 days old.
Fig. 17. Mother sporocyst, 10 days old, optical section of larva drawn as Fig. 16.
Fig. 18. Mother sporocyst, 24 days old.
Fig. 19. Mother sporocyst, 33 days old.
Fig. 20. Mother sporocyst, 33 days old.
Figs. 21–26. Metamorphosis of flame cell in mother sporocyst, side views.
Fig. 27. Giant-flame cell, drawn as Fig. 26, in end view.
Fig. 28. Daughter sporocyst with cercaria emerging from birth pore.

development of large, inactive gorgoderid cercariae has never been described, it will be given here.

In *G. amplicava,* germ cells are located on the lateral and posterior walls of the sporocysts and are similar in appearance to those which occur in the miracidium and mother sporocyst. Germ balls are most evident in the posterior regions of young daughter sporocysts while older stages occur in the middle and anterior regions of the sporocysts. The body of the cercaria possesses *anlagen* of ventral and oral suckers before the tail bud appears. The tail is at first broadly attached to the body and is composed of large vesiculated cells (Figs. 29 and 30); it increases in size at a greater relative rate than the body and early begins to show a central lumen (Figs. 31 and 32). The anterior enlargement is composed of wall cells which are somewhat irregularly polygonal in surface view while the filiform portion of the tail is composed of spherical cells (Fig. 33). When the tail is approximately three times the length of the body, the narrow isthmus connecting the two becomes so attenuated that even gentle handling may break it.

The anterolateral portion of the tail swelling produces a thin cuticula (Fig. 34) which is gradually augmented (Figs. 35 and 36) and which progresses forward to enclose the base of the young distome (Figs. 37, 38, 39, 40). The act of distome retreat into the chamber was not observed and stages between Figs. 40 and 41 were not seen either *in vivo* or in abundant preserved material. Apparently the anterior tail fibers and the conical cell mass (Fig. 40) strained by the anterior growth of the future chamber suddenly retract and pull the distome into the chamber. The cercarial body (Fig. 43) is always detached eventually from the tail but a small cell group at the base of the anterior chamber marks the position of the former tail attachment. The gradual tail distention by vacuolation of the peripheral cells produces the thin-walled, elongate, sac-like tail which is structureless except for a few scattered nuclei, cytoplasmic debris and striae posteriorly, and the conical cell group at the anterior end. The outer tail surface in a newly shed cercaria is glistening, smooth and non-sticky; the larva can be handled conveniently with pipettes. Within an hour or two, however, the tail surface becomes excessively sticky and will cling tenaciously to any object; manipulations of any sort then become difficult. This striking change allows easy discharge from the mollusc and cercarial attachment to aquatic vegetation or other submerged objects.

5. *Metacercaria*

The description of this stage given by Krull (1935) is confirmed by the present study. One excysted metacercaria (Fig. 49) is drawn to show certain histological details not previously reported. The testicular rudiments are already developed in the form of two lateral cords with five swellings of germinal cells on the ovarian side and four similar clusters on the other side. Approximately twelve large germinal cells are contained in the ovary and from two to twelve identical germinal cells

Explanation of Plate 3

Figs. 29–41. Intrasporocyst development of cercariae.
Fig. 42. Cercariae emerging from excurrent siphon of host bivalve (free-hand).
Fig. 43. Anterior portion of cercaria showing chamber with enclosed distome.
Figs. 44–48. Cercarial penetration of tadpole tail (free-hand).
Fig. 49. Metacercaria.
Fig. 50. Excretory bladder cell with excretory concretions.
Fig. 51. Portion of longitudinal nerve cord.

PLATE 3

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in each testicular swelling. The nervous system consists of anterodorsal bilobed cerebral ganglia and two main longitudinal trunks, consisting of ovoid inclusions, 4–5 μ by 2–3 μ, with peripheral granules all surrounded by a sheath possessing nuclei 5 μ in diameter (Fig. 51).

The granular concretions of the excretory bladder of the aging metacercaria are very conspicuous. These have been described as "rosette-shaped" in *Phyllodistomum solidum* by Goodchild (1943); under higher magnification the granules in *G. amplicava* are seen to be separate and to be enclosed in the cytoplasm of cells lining the excretory bladder wall which may be the remnants of the cytogenous gland cells (Fig. 50). As previously pointed out, these granule-laden cells are discharged through the excretory pore during the normal migration of the excysted worm in the posterior regions of the amphibian enteron moreover, pressure on the cover glass of a mechanically excysted metacercaria can dislodge these cells and force them with their concretions to the outside through the excretory pore.

The body cells of the metacercaria, as in other stages of this life cycle, are large, 40–50 μ, vacuolated cells with nuclei, 20 μ in diameter, having single eccentric chromatin granules.

6. *Adult*

Krull (1935) and Odlaug (1937, 1939) traced the development of the adult in the amphibian host while Bensley (1897), Stafford (1902), Guberlet (1920) and Harwood (1932) described the anatomy of the sexually mature adult *G. amplicava*.

A developmental series of the adult *G. amplicava* is given in Figs. 52 through 74. The maturation of the reproductive organs, the gradual postacetabular uterine distention, the changes in general shape and body organs relationships are indicated, as well as the great range in oral to ventral sucker ratios. The smallest adult in this series (Fig. 52) is 0.85 mm long, whereas the largest one (Fig. 74) is 4.00 mm long.

DISCUSSION

Gorgodera amplicava is first intermediate host specific. It develops in *Musculium partumeium,* and fails to infect *M. securis* or *Sphaerium occidentale* in the same microhabitats. This failure is not due to any selectivity on the part of the feeding mechanism of these molluscs because miracidia introduced into the incurrent streams are passively swept into the mantle cavities. The failure to complete development in the non-normal hosts must reside in a fundamental incompatibility between mollusc and miracidium. Martin (1937) stated that *Sphaerium sp.* harbored the larval stages of this fluke. However, the statement is probably a typographical error for *Musculium sp.* because in the same paper he gave incorrectly *Sphaerium partumeium* as Krull's reported bivalve host.

A less rigid larval host selectivity occurs in *Phyllodistomum solidum* which utilizes either of the two species: *Pisidium abditum,* according to Goodchild (1943) or *P. pusillum* (Gmelin), according to Groves (1945) who also reported failure of larval development after entrance in *P. variabile* Prime, *Sphaerium striatinum* (Lamarck), and *Musculium transversum* (Say). My previously unpublished data show that *Musculium partumeium* and *M. securis* could not be infected with miracidia of *P. solidum.*

EXPLANATION OF PLATE 4

FIGS. 52–74. Development of adult *G. amplicava* in frog host.

PLATE 4

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Gorgoderid cercariae are among the most interesting in the whole group of trematodes. The fact that they are produced in lamellibranchs rather than in gastropods, the fact that some are small and microcercous and others are large and macrocercous, the fact that some are active and are eaten by predaceous animals, that some are inactive and are passively ingested, and that some have stylets while others are without stylets make them a unique group. The possible phylogenetic interrelationship and significance of cercarial differences in this group were given by Goodchild (1943) who expressed the probability that, "cercarial structures in the gorgoderids, at least, are only caenogenetic features which have been modified to fit the exigencies of their varied life histories." In addition, further supporting the great adaptability of larval structures in trematodes, adult worms recovered from Missouri bullfrogs, and belonging undeniably to the species *G. amplicava,* produced miracidia which are morphologically unlike those from the Massachusetts adults. This difference is being investigated further.

Gorgoderid life cycle generalizations can now be made. The metacercarial host is almost any animal which ingests the cercaria. Growth and slight differentiation do occur in the metacercarial stage, but are not required because metacercariae are almost immediately infective for the final host. The active gorgoderid cercariae are, in general, eaten by predaceous animals such as odonatan nymphs, caddis worms, aquatic beetle larvae and urodele larvae. The inactive cercariae are normally eaten by tadpoles and snails, but as shown above, other animals are potential hosts. Fish, fresh-water shrimps, crayfish, and adult Amphibia are additional hosts. This list will no doubt be greatly extended with further work in the group.

Certain adult gorgoderids are apparently less host specific than others. *Phyllodistomum folium* has been reported from approximately ten European cyprinids while other species have been found in from three to only one host species. *G. amplicava* can apparently excyst and invade the urogenital system of most anurans and several urodeles including *Siren lacertina* L. according to Bennett and Humes (1938), and *Ambystoma maculatum, Pseudotriton montanus montanus* (Baird) and *P. ruber ruber* (Sonnini) according to Rankin's (1937) review of the literature.

The mechanism of final host immunity is not known. Excystment of metacercariae of *G. amplicava* occurs in almost all the animals fed. Normal posterior migration in the gut and orientation to the cloacal urogenital openings is not achieved in all, however. Goodchild (1945) suggested a mucous barrier in *Triturus v. viridescens.* In the painted turtle, *Chrysemys p. picta* (Schneider), all metacercariae excyst in the small intestine, but they do not achieve any independence of motion and are carried along peristaltically with the chyme until they are finally eliminated. The contents of the digestive tract apparently are deleterious in this instance and constitute an immune barrier.

Adult gorgoderids inhabit the urogenital systems of their hosts. Odlaug (1937) described young *G. amplicava* adults from the kidneys of Massachusetts bullfrogs. Furthermore, he stated, "No worms were found in the ureters." In the present study on bullfrogs from the same region, young *G. amplicava* were not found in the mesonephric tissue; rather, they were confined to the Wolffian ducts or to the seminal vesicles. As many as 50–100 young adults have been recovered from the mesonephric ducts of both male and female frogs. These worms were concentrated at the upper end of the ducts which are often slightly swollen by the masses of them packed

there, and, in the male, large numbers were located in the spongy seminal vesicles. The newly excysted adult can invade the bladder directly but apparently the majority seek out and enter the excretory ducts where they remain for at least two weeks.

The development of the male reproductive system agrees closely with the account given by Krull (1935) who stated, "In stained and mounted specimens the dense testicular mass shows little evidence of being subdivided into testes, and there is little separation into right and left parts until maturity." This statement does not imply that there is any change in the number of testicular units as reported by Odlaug (1937) or Rankin (1939); in fact, nine testicular masses have been traced, in the present study, from the cercarial stage through the sexually mature adult. The maturing adult usually has nine testes, and in flattened specimens the division into left and right halves was always apparent. Anteroposterior separation of the gonads was accomplished by inter-testicular infolding of egg-bearing uterine coils. Exceptional specimens rarely occur: for example, Fig. 68 possesses a single testis anterior to the ovary, while Fig. 53 and Fig. 61 have only eight testes; in the latter, moreover, three testes are located on the ovarian side and five are on the side opposite.

Gorgodera minima Cort, 1912, from *Rana catesbeiana* and *R. pipiens,* is the only other recognized North American species of *Gorgodera.* Cort erected the species on the basis of small size (1.58 mm long), sucker ratio diversities, differences in the shape and relations of the reproductive organs, a cylindrical postacetabular body, and a relatively larger seminal vesicle. Sucker ratio ranges are of little specific significance in the gorgoderid group, e.g., the ratios vary from 1 : 1.7 to 1 : 2.8 in the specimens on Plate 4. Shapes and relations of the reproductive organs are likewise of little taxonomic significance. These qualities are constantly changing and a relatively larger seminal vesicle in young protandric adults is to be expected. For example, the seminal vesicle in Fig. 57 is one-seventh the body length, whereas in Fig. 74 it is one-seventeenth the body length. The Looss' shaking method of fixation used by Cort allows more contraction than the cover glass pressure method employed here. However, Fig. 57 (1.09 mm) and Fig. 58 (1.53 mm) are actually shorter than Cort's specimens. The postacetabular body of a young specimen of *G. amplicava* is cylindrical and blunt, but with growth the relatively broad and tapered body is attained. It is obvious that *Gorgodera minima* was established on the basis of non-existent differences between it and young specimens of *G. amplicava.*

The similarities between *G. minima* and young *G. amplicava* are nearly as striking as the lack of differences. The testes and vitelline follicles in both are identical as regards shape, arrangement, and numbers. The distributions of the uteri in Cort's specimens on deposit in the United States National Museum and in his Figs. 1 and 2, are the same as shown in my Figs. 58, 59 and 60. Final clenching proof of their identity is obtained from Cort's specimens and Figs. 1, 2 and 3 where more or less three-lobed ovaries, a specific character featured in all descriptions of *G. amplicava,* occur regularly. *G. minima* must, therefore, be suppressed as a synonym of *G. amplicava.*

SUMMARY

A study of the life history and bionomics of *Gorgodera amplicava* Looss, 1899, from Cape Cod, Massachusetts, and southwestern Missouri, has been made.

The sphaeriid bivalve mollusc, *Musculium partumeium,* taken from the same

natural habitats as *M. securis* and *Sphaerium occidentale* has alone been found naturally infected, while *M. partumeium* used in successful laboratory controlled miracidial experiments has been maintained in continuous laboratory cultures for four years.

Cercariae were ingested with food or in respiratory currents into tadpoles of several anuran species including: *Rana catesbeiana, R. clamitans, R. palustris* and *Hyla versicolor,* and these amphibians were all satisfactory natural and experimental metacercarial hosts. Moreover, several species of snails, *Pseudosuccinea columella* and *Physa heterostropha* were additional natural and experimental metacercarial hosts.

Penetration and early encystment of metacercariae have been obtained in the larval urodele, *Ambystoma maculatum* and the odonatan nymph, *Enallagma sp.*

Encystment *in vitro* and ectopically in tadpole tails was accomplished.

Metacercarial excystment and successful invasion of the urogenital systems (i.e., urinary bladder, mesonephric ducts or oviducts) occurred in all anuran final hosts tried, including: *R. catesbeiana, R. clamitans, R. palustris, R. pipiens brachycephala, R. pipiens sphenocephala* and *Bufo americanus.*

Ambystoma maculatum is a suitable experimental urodelan final host, while *Triturus v. viridescens* is not. In the latter, normal excystment occurs but the young worms become trapped in fecal pellets and are eliminated.

In the painted turtle, *Chrysemys p. picta,* metacercariae excyst in the small intestine but do not achieve independence of motion and are carried along peristaltically with the chyme until they are eliminated.

All stages in the life cycle have been studied and described or figured.

The miracidia are approximately 55 μ long by 28 μ wide and are covered with fifteen epidermal plates arranged in three rows. The anteroposterior pattern is 6, 6, 3. An apical adhesive and a pair of penetrating glands as well as two flame cells, germ cells and body cells are found internally.

Experimentally produced mother sporocysts, located in the gills of the bivalve, have been traced from miracidial penetration to the liberation of daughter sporocysts. It has been found that the miracidial epidermal plates persist as a loose, intact investing sporocyst cover for as long as 43 days after miracidial penetration.

A unique flame cell metamorphosis has been found in mother and daughter sporocysts and in the adult.

The large-tailed cercaria of *G. amplicava* agrees closely with the published description,but has been found to be much longer than previously reported, with an average length of 13.9 mm (12–17.4 mm) for recently shed cercariae.

The intrasporocyst development of the cercaria has been traced and a minutely graded developmental series of the adult in the frog host is given.

Gorgodera minima Cort, 1912, is shown to have been established on the basis of non-existent differences between it and young specimens of *G. amplicava* and is, therefore, suppressed as a synonym of *G. amplicava.*

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STUDIES ON GERMINAL DEVELOPMENT IN REDIAE OF THE TREMATODE ORDER FASCIOLATOIDEA SZIDAT, 1936[1]

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INTRODUCTION

In a program of investigations on the embryology of the digenetic trematodes which has been carried on for several years at the University of Michigan Biological Station, studies have been made of representatives of the strigeids (Cort and Olivier, 1941), the plagiorchiids (Cort and Olivier, 1943a; Cort and Ameel, 1944), and the schistostomes (Cort and Olivier, 1943b; Cort, Ameel, and Olivier, 1944). Multiplication of individuals in the mother and daughter sporocysts of these groups has been found to be both by direct division and separation of the germinal cells (primary polyembryony of the original zygote), and by a secondary polyembryony of germinal masses.[2] These germinal masses are peculiar structures, composed both of unicellular and multicellular components. In the strigeid and plagiorchiid sporocysts the germinal masses are not attached to the body wall but float freely in the body cavities mixed with the developing embryos. From them break off the largest multicellular components to continue development into daughter sporocysts or cercariae. Since the germinal masses are constantly giving off embryos throughout the life of the sporocysts, it is evident that some of their unicellular components (germinal cells) must divide to form new germinal cells over a long period of time. These germinal masses in the strigeids and plagiorchiids can be interpreted, therefore, as floating centers of persistent multiplication of germinal cells, which bring about the production of the extraordinarily large numbers of individuals produced in the germinal sacs of these groups. The important thing, then, is not so much the presence in these germinal masses of small embryos (multicellular components), as the ability of some of their germinal cells (unicellular components) to continue dividing for such a long time.

Since all the work reviewed above on the development in the germinal sacs of the digenetic trematodes was done on groups with daughter sporocysts in their life cycles, we decided in the summer of 1947 to devote most of our attention to the development of the germinal material in rediae. Descriptions and figures of rediae in the literature give a very confusing picture of the germinal material. In most cases, embryos in various stages of development are shown inside the rediae with the smallest ones usually near the posterior end of the body cavity. Also, in some cases, either small masses of germinal material or separate germinal cells are shown just at the posterior tip of the body cavity, sometimes attached to the wall.

Brooks (1930) described and figured "germ-masses" in rediae belonging to several different trematode groups. His studies were all made on sections of fixed material, mostly of mature rediae. It is a little difficult to understand some of his figures and descriptions, and to follow his interpretations. On the other hand, in

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[1] A joint contribution from the University of Michigan Biological Station, the School of Hygiene and Public Health, the Johns Hopkins University, and Kansas State College.

[2] The term germinal mass is suggested as a better name for these structures than the term "germ-mass" proposed by Brooks (1930), which was used in the earlier papers.

three recent detailed studies of the germ cell cycles of the digenetic trematodes in groups with rediae (Cable, 1934; Chen, 1937; Rees, 1940), the authors noted specifically that no "germ-masses" were present in the rediae. It was the failure of these workers to find germinal masses that led the senior author to make the following statement in a recent paper (Cort, 1944, p. 279). "It can possibly be suggested, therefore, that in groups with rediae as secondary germinal sacs in which rather limited numbers of cercariae are produced, the multiplication of individuals may be entirely by direct division of the cells of the germinal line." We were, therefore, quite surprised to find that germinal masses, in some cases of considerable size and complexity, are present in rediae belonging to several different families of the digenetic trematodes.

The present paper will be limited to the studies during the summer of 1947 of rediae of species belonging to the order FASCIOLATOIDEA Szidat, 1936. In this order we had available for study rediae of the families PARAMPHISTOMIDAE, NOTOCOTYLIDAE, PSILOSTOMIDAE, and ECHINOSTOMIDAE. A brief abstract of the results of these investigations has already been published (Cort, Ameel, and Van der Woude, 1947).

Szidat (1936) pointed out the similarity between the life cycles and larval stages of the PARAMPHISTOMIDAE and NOTOCOTYLIDAE and placed them in the suborder PARAMPHISTOMATA, under the order FASCIOLATOIDEA. In the next year this same author (1937) called attention to the fundamental similarity in structure and life cycles of the PSILOSTOMIDAE and the ECHINOSTOMIDAE, and later (Szidat, 1939) set up the suborder ECHINOSTOMATA, also under the order FASCIOLATOIDEA, to include the ECHINOSTOMIDAE, PSILOSTOMIDAE, and FASCIOLIDAE. These revisions in classification are based largely on similarities in life cycles and larval stages, and seem to us to be an important step in the building of a more natural classification for the digenetic trematodes.

In the order FASCIOLATOIDEA the pattern of the life cycle and the structure of the larval stages seem to be more primitive and generalized than in other trematode groups. In general, the miracidia develop and hatch in the open. The mother sporocysts are small simple sacs, which appear less specialized than the larger more complex mother sporocysts of certain other trematode groups. The rediae are for the most part well developed, with a large intestine, collar and locomotor appendages. It seems probable that the sac-like rediae with reduced intestine and the daughter sporocysts of other groups are derived from this more generalized type of secondary germinal sac. In fact, as pointed out by Brooks (1930), a whole series of transitional stages from well differentiated rediae to very simple sac-like daughter sporocysts is found in different trematode groups. Another point that suggests primitiveness in this order is the large size of the bodies of the cercariae and the extent of development in them of adult characters. This is most striking in the cercariae of the amphistomes and notocotylids which complete their development outside the rediae in the tissues of the snail. In these groups also, the presence of eye-spots and pigment suggest relationship to free-living ancestors. Also, it seems almost certain that the introduction of a second intermediate host into the life cycle of the digenetic trematodes was a purely secondary adaptation for better transportation to the definitive host. In the FASCIOLATOIDEA, when second intermediate hosts are present, as in the psilostomes and echinostomes, the relation

of the cercariae and metacercariae to them is primitive since the cercariae have no special larval adaptations for penetration, and, with only a very few exceptions, the metacercariae do not feed and grow. Finally, in this order the numbers of individuals produced in the germinal sacs is small compared with most other trematodes. This is particularly true in the PARAMPHISTOMIDAE which may perhaps be considered the most primitive of all the digenetic trematodes for which we have life cycle information. Szidat (1939) suggested that the NOTOCOTYLIDAE were derived from this family and pointed out as evidence of its great age that it has representatives in all classes of vertebrates.

The considerations discussed above make it of special interest to obtain information on the germ cell cycle of representatives of the order FASCIOLATOIDEA. Here we would expect the mechanism of development in the germinal sacs to be simpler and more generalized than in such specialized groups as the plagiorchiids, schistosomes and strigeids, in which the number of individuals produced is so very much greater.

In the summer of 1947 we had available for study from examinations of natural infections in snails in the vicinity of the University of Michigan Biological Station rediae belonging to four families of the order FASCIOLATOIDEA. As would be expected no mother sporocysts were found in these infections, although for some of the species both mother and daughter rediae were obtained. Except for some of the echinostomes, specific identifications were not difficult on the material studied on account of previous life history studies. The following outline gives the position of the species studied in Szidat's classification. They will be discussed in the order in which they are numbered in this outline.

Classification of species studied

Order FASCIOLATOIDEA Szidat, 1936
- Suborder PARAMPHISTOMATA Szidat, 1936
  - Family PARAMPHISTOMIDAE Fischoeder, 1901
    1. *Allassostoma parvum* Stunkard, 1916
  - Family NOTOCOTYLIDAE Lühe, 1909
    2. *Notocotylus urbanensis* (Cort, 1914)
- Suborder ECHINOSTOMATA Szidat, 1939
  - Family PSILOSTOMIDAE Odhner, 1911
    3. *Psilostomum ondatrae* Price, 1931
  - Family ECHINOSTOMIDAE Looss, 1902
    4. *Echinostomum revolutum* (Froelich, 1802)
    5. Echinostome from *Physa* sp.
    6. Echinostome mother rediae from *Helisoma trivolvis* (Say)
    7. Echinostome from *Lymnaea stagnalis appressa* Say
    8. *Petasiger nitidis* Linton, 1928

MATERIAL AND METHODS

All the studies recorded in this paper were made on natural infections of various ages in snails collected near the University of Michigan Biological Station. In the examination for larval trematodes the bodies of the snails were carefully removed from the shell in normal saline in Syracuse watch glasses, and teased apart with dis-

secting needles under a low power binocular microscope. Rediae of various ages, when found, were mounted in normal saline and studied alive under the highest available magnifications. In fact, most of the observations on the germinal material in the rediae were made with oil immersion objectives. The findings wherever possible were repeatedly checked by all three of the authors, and in most cases the studies on a particular species were repeated on different infections at different times during the summer. The drawings are free hand sketches, and the measurements are as close an approximation as was possible with the type of living material used.

FAMILY PARAMPHISTOMIDAE

1. *Allassostomum parvum* Stunkard, 1916. Beaver (1929) demonstrated by experimental infections that a cercaria found in *Helisoma trivolvis* (Say) near Urbana, Illinois belonged to the turtle amphistome species, *A. parvum*. We identified as the larval stages of this species, an amphistome infection found in one out of 129 specimens of *Helisoma campanulatum smithii* (Baker) which were collected from a small lake (Round Lake) close to Twin Lakes about 20 miles southeast of the Biological Station. This infection was mature and contained a number of developing cercariae in the snail's tissues, a small number of mature mother rediae, and daughter rediae in various stages of development.

We studied the germinal material in four small mature mother rediae, which were between 0.50 and 0.76 mm in length. They contained only a small number of daughter redial embryos, which, however, filled their body cavities completely and were crowded against each other. The smallest embryo in each redia was at the posterior end of the body cavity, and the largest, which had a well developed pharynx and intestine, and appeared ready to escape, was at its anterior end. In the mother redia shown in figure 1 there were three more daughter redial embryos in front of those shown in the figure making a total of 13. In another, 0.50 by 0.12 mm, there were 10 embryos in all. Each mother redia had a simple germinal mass at the posterior end of its body cavity attached to the wall, which appeared to be composed entirely of unicellular components (Fig. 1). The number of components in these germinal masses was few, in one mother redia there being only six or eight. The smallest free embryos next to the germinal masses were little if any larger than their components, and it was not possible to be sure whether they were detached germinal cells or very early embryos.

Daughter rediae were present in this infection in various stages of development. At least six very young daughter rediae were carefully studied. They had lengths from 0.24 to 0.37 mm according to the state of contraction. In two, there were no free embryos and in the others there were from three to five small embryos wedged between the posterior end of the intestine and the anterior end of the germinal mass. The germinal masses in these very young daughter rediae consisted of slightly elongate morula-like groups of unicellular components, firmly attached at the posterior tip of the body cavity (Figs. 2 and 3). The smallest free embryos were somewhat larger than the components of the germinal mass and appeared to have more than one cell.

At a slightly older, but still immature, stage (Fig. 4), the number of free embryos had increased and the anterior one was quite large although still far from ready to escape. The germinal mass was smaller and the number of its com-

ponents less. In another immature daughter redia in which eight or nine free embryos could be counted the germinal mass was still smaller, appearing to have only 8 to 10 unicellular components.

In several young mature daughter rediae in which the largest cercarial embryos were well differentiated and apparently ready to escape, the number of components in the germinal masses was still further reduced (Fig. 5).

In this species, therefore, the germinal mass of both the mother and daughter rediae appears to consist of a group of germinal cells joined together in a morula-like mass attached at the posterior end of the body cavity. The embryos seem to be formed from components detached from the anterior part of this mass. In those rediae which are filled with embryos, the number of components in the germinal masses is reduced.

FAMILY NOTOCOTYLIDAE

2. *Notocotylus urbanensis* (Cort, 1914). We identified as belonging to *N. urbanensis,* notocotylid cercariae found in *Physa* sp. (probably *P. gyrina* Say), obtained from a pond in Wilderness Park about 20 miles north of the Biological Station. Information on the life cycle of this species in this region has been given by Luttermoser (1935) and Herber (1940). Twelve infections were found during the summer in three collections totaling 462 snails. In only one of these were mother rediae found. Eight others containing only daughter rediae were studied at different times during the summer giving ample opportunity for the checking of observations.

PLATE I

DESCRIPTION OF FIGURES

FIG. 1. Posterior part of mother redia of *Allassostoma parvum,* 0.60 by 0.15 mm, showing germinal mass and embryos in different stages of development; three more redial embryos were present in this mother redia, the largest showing a differentiated pharynx.

FIG. 2. Posterior part of very immature daughter redia of *A. parvum,* 0.37 by 0.07 mm, showing germinal mass composed of unicellular components attached at the posterior end of the body cavity; no free embryos are present.

FIG. 3. Very immature daughter redia of *A. parvum,* 0.32 by 0.07 mm, showing germinal mass and three free embryos.

FIG. 4. Immature daughter redia of *A. parvum,* 0.42 by 0.10 mm, showing germinal mass attached at posterior end of body cavity and graded series of embryos.

FIG. 5. Posterior end of mature daughter redia of *A. parvum,* 0.64 by 0.15 mm, showing a reduced germinal mass in the posterior end and embryos of various sizes.

FIG. 6. Mature mother redia of *Notocotylus urbanensis,* 0.70 by 0.30 mm, showing germinal mass attached to wall near posterior end, and series of daughter redial embryos.

FIG. 7. Very immature daughter redia (redial embryo ?) of *N. urbanensis,* 0.30 by 0.12 mm, showing germinal mass composed of unicellular components attached by fibers to walls of body cavity near posterior end; the intestine appears rudimentary and no free embryos are present.

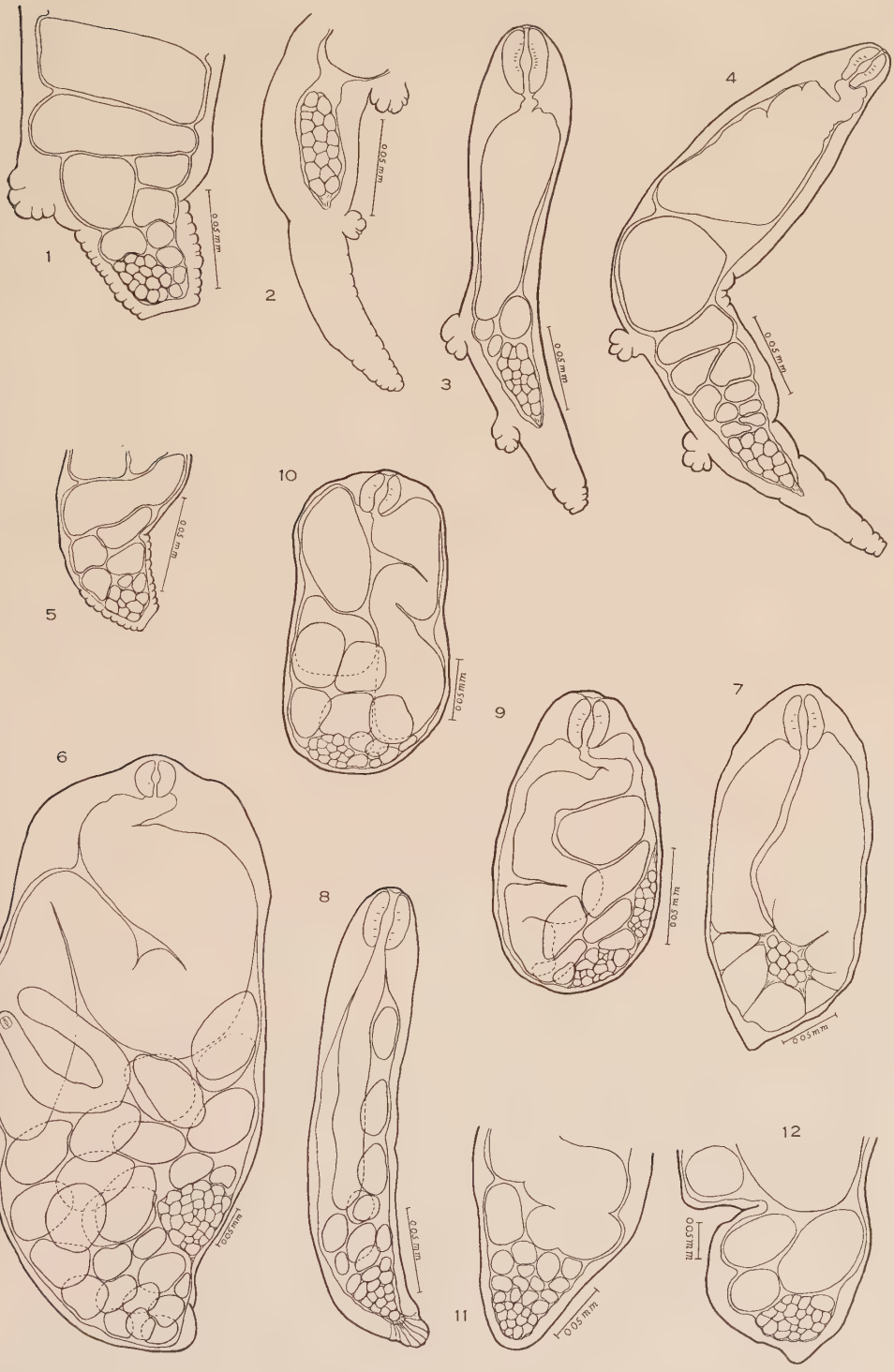
FIG. 8. Daughter redial embryo of *N. urbanensis,* 0.28 by 0.05 mm, showing germinal mass attached at posterior end, and a series of small developing embryos.

FIG. 9. Immature daughter redia of *N. urbanensis,* about 0.17 mm long, showing what appears to be a divided germinal mass attached to the wall of the body cavity at the posterior end and along one side.

FIG. 10. Immature daughter redia of *N. urbanensis,* about 0.26 mm long, showing a small germinal mass at the posterior end and a series of embryos in the body cavity.

FIG. 11. Posterior end of immature daughter redia of *N. urbanensis,* about 0.30 mm long, showing small germinal mass and series of small embryos.

FIG. 12. Posterior end of old heavily pigmented daughter redia of *N. urbanensis,* 0.66 by 0.24 mm, showing a fairly large germinal mass still persisting in the posterior end of the body cavity.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In the snail in which mother rediae were found there were also some immature daughter rediae. All the other infections contained mature and, not infrequently, old daughter rediae. In them also were varying numbers of immature daughter rediae, some of which appeared to have but recently escaped from the mother. Although no mother rediae were found in any of these eight infections it is not improbable that they were present, but missed, because of the large numbers of daughters present. At least the presence of very immature stages in infections with mature and old daughter rediae suggests that mother rediae of this species persist for a considerable time and continue to give off daughters. It is probable that these infections had all lasted over the winter. In fact, in as late a season as the spring of 1947 it seems impossible that infections acquired in that season could have become mature by the time we made our collections (Cort et al, 1937).

We made counts of the daughter rediae in six of the snails infected with *N. urbanensis,* which gave the following numbers: 104, 132, 208, 237, 352, 412, averaging 289. It is probable that our counts were too small especially where large numbers of very small immature rediae were present. At any rate they show that considerable numbers of daughter rediae can be produced by the small number of mothers that is characteristic of this species. Herber (1940) reported 6 to 8 mother rediae as the characteristic number produced in the mother sporocysts of *N. urbanensis.*

In the one infection with mother rediae three were found. There was also a number of young immature daughters, but none that approached maturity. Two of these mother rediae were carefully studied and the other one was broken and some study was made of daughter redial embryos from it. One of these mothers measured 0.70 by 0.30 mm (Fig. 6) and the other 0.96 by 0.36 mm. Both had very voluminous intestines and numbers of daughter redial embryos in various stages of development in their body cavities. One embryo in each had a differentiated pharynx and digestive tract and appeared ready to escape. In each of these mother rediae a germinal mass was found attached to the wall at one side near the posterior end (Fig. 6). It was comparatively large and contained numbers of both unicellular and multicellular components.

The germinal material was studied in a few daughter redial embryos, each containing several free cercarial embryos, taken from these mothers. The germinal masses at this stage are attached at the posterior end of the body cavity and seem to be composed only of unicellular components. An immature daughter redia was found in which there were no free cercarial embryos, and with what appeared to be a rudimentary intestine (Fig. 7). It was probably an embryo broken out of a mother redia although no mothers were found in this infection. At least it contained no free embryos and its germinal mass was composed of unicellular components and was attached by strands to the wall of the posterior end of the body cavity.

A large series of very immature daughter rediae varying in length from 0.16 to 0.30 mm was examined at different times during the summer. Typical examples are shown in Figs. 9, 10, and 11. Part of these had rather attenuated posterior tips. In these the germinal mass appeared as a group of unicellular components attached at the posterior end of the body cavity, with the smallest embryos just in front of it (Fig. 11). In some cases the youngest daughter rediae had rounded posterior ends and were much thicker in relation to their width. In these, the

germinal mass appeared to consist only of unicellular components, and was attached either at the posterior tip of the body cavity or to one side of it (Fig. 10). In all cases the smallest embryos were close to the germinal mass. Figure 9 represents an exception, the only one which was found, in which the germinal mass was scattered in two parts along the wall and appeared to have multicellular components adhering to it. However, small germinal masses consisting only of unicellular components seemed to be typical of these small daughter rediae.

We also studied quite a series of larger immature rediae varying in length from about 0.36 to 0.56 mm. They were unpigmented and the largest cercarial embryos in them had not yet reached the stage when they were ready to escape. In all of them the germinal mass was attached at the posterior end of the body cavity and consisted of unicellular components. In most instances the number of its components was distinctly less than in the youngest daughter rediae, although there were a few that had germinal masses about the size of that shown in Fig. 11. Some of these young rediae had the germinal mass reduced to about half a dozen components, and in a few they appeared reduced even below that number (Fig. 15). It appears, therefore, that in some of the daughter rediae even before maturity the germinal mass may be reduced to a few components by the separation of embryos.

Finally, studies were made of a number of mature and old daughter rediae of *N. urbanensis*. They varied greatly in size, the smallest being 0.66 mm by 0.24 mm and the largest 2.1 by 0.17 mm. Their usual length was about 1 mm. They also varied greatly in regard to their germinal masses. In some, the masses appeared as large as those in younger rediae (Fig. 12) while in others none could be found (Fig. 16). In others, the numbers of components showed all variations between these two extremes (Figs. 13, and 14).

In the rediae of this notocotylid, therefore, we found two kinds of germinal masses. In the mother rediae they appear to be rather large and persistent with both unicellular and multicellular components, similar to those of the psilostome and echinostome species described later. This may perhaps be associated with the fact that small numbers of mother rediae produce a considerable number of daughters. On the other hand the germinal mass in the daughter rediae of this species is like that of the amphistome, *A. parvum,* with only unicellular components which are reduced in number by the production of cercarial embryos. As in the amphistome this type of germinal mass in *N. urbanensis* appears to be associated with the production of a comparatively small number of cercarial embryos. However, there seems to be in the daughter rediae of this species a considerable variation in the stage of development in relation to the reduction in number of components of the germinal mass. In some cases it is almost entirely used up in immature rediae, while in others germinal masses with a considerable number of components are still present in mature rediae. This would appear to be due to variations in the time during which the germinal cells retain the power of dividing.

FAMILY PSILOSTOMIDAE

3. *Psilostomum ondatrae* Price, 1931. McMullen (1938) described the larval stages of *P. ondatrae* and Beaver (1939a) worked out its life cycle experimentally in the Douglas Lake region. In the summer of 1947 we found the laval stages of this species in *Helisoma trivolvis* (Say) is 10 out of 335 examinations, and in

H. antrosum percarinatum (Walker) in 18 out of 253 examinations. We, therefore, had ample material for study at several times during the summer. Both the cercariae and rediae of this species are very characteristic and easy to identify.

Mother rediae of *P. ondatrae* were found in five snails. The numbers recorded from three of these infections being 5, 20, and 32. In none of them had daughter rediae escaped from the mothers. Some of these mother rediae were large and contained large numbers of embryos in various stages of development. One, 1.5 by 0.25 mm contained a total of 131 embryos; a second, 1.6 mm long, contained 80; and a third, 1.7 by 0.20 mm, had a total of 77. These daughter redial embryos varied from those that had a well developed pharynx and intestine with several cercarial embryos already present in the body cavity, to very small "germ balls" of only a few cells.

Counts were made of daughter rediae in a series of mature infections. In eight infected specimens of *H. antrosum percarinatum,* in which the majority of the daughter rediae were mature or old, their numbers varied from 260 to 606 with an average of 434. These counts are probably too low because the presence of immature rediae made an accurate count difficult. The snails were rather

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PLATE II

DESCRIPTION OF FIGURES

FIG. 13. Posterior end of mature daughter redia of *N. urbanensis,* about 1.4 mm in length, showing small germinal mass attached to wall at posterior end; note that some of the smaller embryos are also attached to the wall by strands.

FIG. 14. Posterior end of mature daughter redia of *N. urbanensis,* 1.04 by 0.27 mm, showing small germinal mass at one side at posterior end and series of embryos.

FIG. 15. Posterior end of immature daughter redia of *N. urbanensis,* 0.56 by 0.22 mm; the only remnant of the germinal mass left appears to be a few germinal cells attached to the posterior end of body cavity.

FIG. 16. Posterior end of old daughter redia of *N. urbanensis,* 0.95 by 0.24 mm; no germinal mass could be found suggesting that it may have been completely used up in the production of embryos.

FIG. 17. Mature mother redia of *Psilostomum ondatrae,* about 0.75 mm long, showing a large germinal mass composed of both unicellular and multicellular components attached at one side near the posterior end of the body cavity; a large number of free embryos in various stages of development are present.

FIG. 18. Posterior end of large mature mother redia of *P. ondatrae,* about 1.6 mm long, showing complex germinal mass attached to one side near the posterior end; about 80 redial embryos were counted in this mother varying from those no larger than the largest multicellular components of the germinal mass to well developed stages ready to escape.

FIG. 19. Posterior end of large mature mother redia of *P. ondatrae,* 1.7 by 0.2 mm, showing a complex germinal mass attached at one side near posterior end; in all, 77 embryos were counted in this redia, six of which had a well developed pharynx; the smallest free embryos are about the same size as the largest multicellular components of the germinal mass.

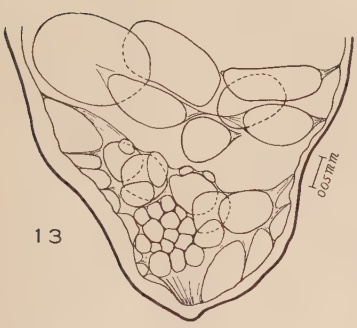
FIG. 20. Posterior end of very immature daughter redia of *P. ondatrae,* 0.24 by 0.03 mm, showing a large germinal mass composed of both unicellular and multicellular components attached at posterior end of body cavity and extending forward almost to the posterior end of the intestine; no free embryos are present.

FIG. 21. Posterior end of very immature daughter redia of *P. ondatrae,* 0.19 by 0.04 mm, showing germinal mass and very small embryos at posterior end of body cavity.

FIG. 22. Immature daughter redia of *P. ondatrae,* 0.58 by 0.08 mm, showing germinal mass and a number of immature cercarial embryos.

FIG. 23. Posterior end of old daughter redia of *P. ondatrae,* 1.8 by 0.25 mm. showing large complex germinal mass flattened against the wall of the posterior end; many mature cercariae are present and also a series of immature embryos the smallest of which are no larger than the largest multicellular component of the germinal mass.

FIG. 24. Posterior end of mature daughter redia of *P. ondatrae,* 0.79 by 0.16 mm, showing much flattened germinal mass at one side of posterior end.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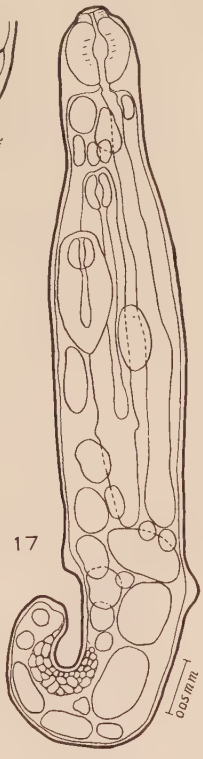
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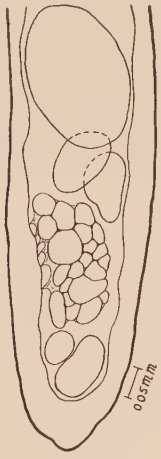
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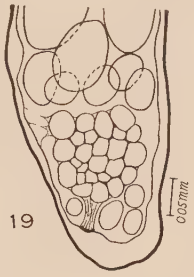
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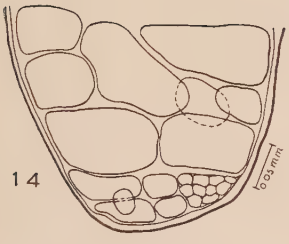
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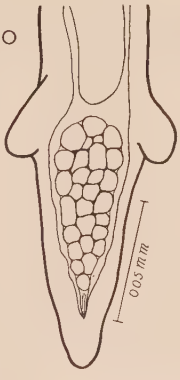
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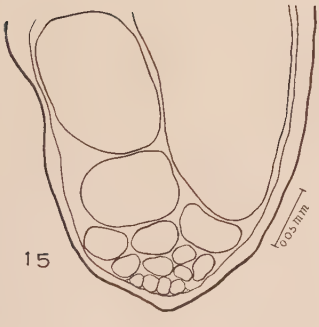
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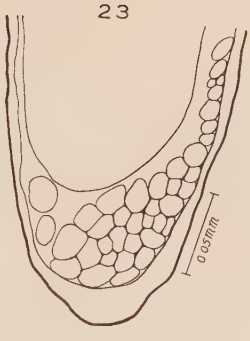
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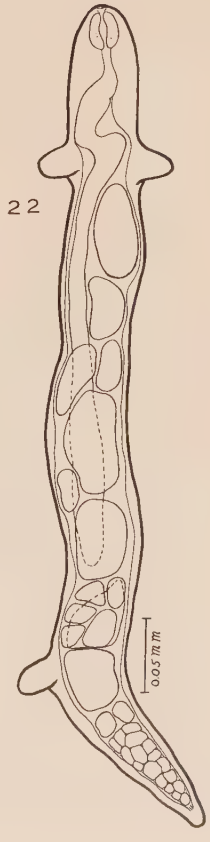
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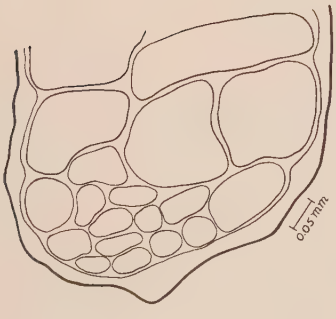
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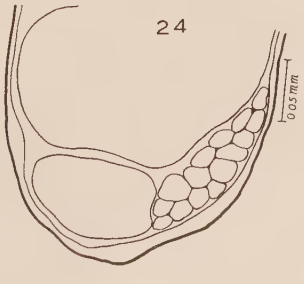
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small, having a shell diameter of about 10 to 12 mm. The daughter rediae were also counted in two large specimens of *H. trivolvis,* 24 and 25 mm in diameter. In one, 2677 mature daughter rediae were counted, and in the other the count was 1432; the few immature ones present in these infections were not counted. No counts were made on cercarial production by daughter rediae but usually several fully developed cercariae as well as large numbers of developing embryos were present in a single daughter redia. Also, the daughter rediae grew to large size since some were from 1.5 to 2 mm in length. Most mature daughter rediae, however, were about 1 mm in length. While accurate comparisons are not possible it seems evident that the production of individuals is considerably larger in this species than in *A. parvum* and *N. urbanensis.* Also, it is interesting that even in the oldest infections there were immature daughter rediae, frequently of the smallest size, suggesting that the mother rediae live for a considerable time and continue to produce young. This indicated persistence and active reproduction of mother rediae for considerable periods of time since in this case, also, it seems almost certain that all the infections containing mature daughter rediae had been in the snails over the winter.

All the mother rediae that were studied had large germinal masses attached at one side of the body cavity near the posterior end (Figs. 17, 18, and 19), composed of both unicellular and multicellular components. They were strongly attached and very stable structures, as is shown by the extent to which they were flattened and pushed around without being displaced or broken up by the movements of the embryos and the extension and contraction of the mother rediae. The largest multicellular components of these germinal masses were about the same size as the smallest free embryos (Figs. 18 and 19). It seems evident that these germinal masses of the mother rediae of *P. ondatrae* are persistent centers of multiplication of the cells of the germinal line. Their large size and persistence can be associated with the rather large numbers of embryos of second generation rediae which they produce.

Daughter redial embryos of this species, having a length when extended up to almost 0.5 mm and containing several free embryos, were found within the mothers. Also, free daughter rediae measuring from 0.19 to 0.36 mm, were present, some of which contained no free embryos. While no mothers were found in the infections with these smallest rediae, it is possible that they may have been present, and that these smallest rediae had broken out of them. The germinal mass at this stage was quite large and filled all or most of the space in the body cavity back of the posterior locomotor appendages (Figs. 20, 21, and 22). It had a large number of components, both unicellular and multicellular, and was firmly attached at the posterior tip of the body cavity. Its largest multicellular components were about the same size as the smallest of the free embryos.

A series of daughter rediae varying from almost mature to old individuals with lengths from 0.6 to 2.0 mm were also studied. In all of these, the posterior ends were broader, and mature, or almost mature, cercariae were present. The germinal masses of all these rediae were much alike consisting of both unicellular and multicellular components. They were flattened against the wall, being attached to one side of the posterior end of the body cavity. Figures 23 and 24 show such germinal masses with the bodies of large cercarial embryos in contact with them.

In old heavily pigmented daughter rediae these flattened germinal masses containing a considerable number of components could still be found. Also, in such rediae small embryos were present.

It appears, therefore, that in this species the germinal masses of both mother and daughter rediae are persistent centers of multiplication with both unicellular and multicellular components which continue to produce new embryos throughout the whole life of the rediae.

THE ECHINOSTOMIDAE

Some of the echinostome material which we studied offered a very difficult problem of identification. The most abundant species was determined from the number and arrangement of the collar spines of the cercaria and metacercaria to be *Echinostoma revolutum*. Also, we were able to identify easily the very characteristic large-tailed cercariae of *Petasiger nitidus*. We could not make precise identifications of the other forms which were studied.

4. *Echinostoma revolutum* (Froelich, 1802) : This common echinostome, which has 37 spines on its collar, was found in abundance in *Helisoma trivolvis* and *H. antrosum percarinatum* (Walker) from the pond in Wilderness Park. In three collections made from July 4 to July 19, 102 out of 740 snails harbored this species. It was common in both the largest snails and in the medium sized ones. The infections were all mature and in most of them the rediae were heavily pigmented. They all appeared to be old infections that had been carried over the winter in the snails. In fact the season was so late in 1947 that spring infections, no matter how early acquired, would not have had time to mature by July 4, when our first collections were made. In many of these infections, even in those that appeared the oldest, very small immature rediae were found suggesting that mother rediae had been recently present or were still present. However, no mother rediae were found in any of the numerous infections examined.

The infections were in *H. antrosum percarinatum,* which were medium sized snails about 10 to 12 mm in diameter, and large old specimens of *H. trivolvis,* about 25 mm across the shell. The daughter rediae were counted in nine of the medium size snails which showed numbers varying from 175 to 540 with an average of 360. In counts of daughter rediae from seven of the large snails the numbers varied from 558 to 3960 with an average of 1724. These counts are all probably somewhat below the true numbers because in such heavy infections it is easy to miss some of the smallest immature rediae.

Studies were made on the germinal material of daughter rediae of *E. revolutum* from six or more of these infections on six different days at various times during the summer. This provided abundant opportunity for repeated checking of our observations.

A study was made of a considerable number of the youngest rediae in which there were only a very few cercarial embryos free in the body cavity. Figure 25 shows the only immature redia examined which had no free embryos. Its germinal mass had both unicellular and multicellular components. This was even more clear in a few that had one to three free embryos. Fig. 28 in which there is only one free embryo illustrates this and gives an unusual view of the attachment of a germinal mass. The large germinal mass shown in Fig. 26 is characteristic of the stage with a larger number of small embryos. Figs. 27 and 29 show germinal

masses in immature rediae in which some of the free embryos are of considerable size. It is characteristic of young echinostome rediae that growth is so rapid that even in immature stages, embryos of various sizes become mixed in the body cavity. As for example, in Fig. 29, the largest embryo present is in contact with the germinal mass. This is in contrast with the condition in the rediae of *A. parvum* where the embryos were packed closely together with the largest at the anterior end of the body cavity, and the smallest next to the germinal mass. In older rediae of *E. revolutum,* which are still not mature, the germinal mass appeared attached at one side of the body cavity and the larger embryos pushed between it and the body wall as the rediae extended and contracted (Figs. 30 and 31). In mature rediae the germinal mass tended to be more flattened (Fig. 32). Even in the oldest infections small germinal masses could be made out in some of the very old heavily pigmented rediae. They were flattened at one side of the posterior tip and contained both unicellular and multicellular components. In mature rediae with mature cercariae these germinal masses could be seen to be put under pressure and to be pushed around by cercariae moving in the posterior end. They appeared to stand an extraordinary amount of mauling without being displaced or broken.

5. Echinostome from *Physa sp.* In the large series of *Physo sp.* examined from the pond in Wilderness Park we found three infections containing only the mother rediae of what appeared to be an echinostome species. These rediae were large, and mature individuals contained large numbers of daughter rediae. They had one very distinct peculiarity in structure in that the intestine was very small,

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PLATE III

DESCRIPTION OF FIGURES

FIG. 25. Very immature daughter redia of *Echinostoma revolutum,* about 0.24 mm in length, showing germinal mass composed of both unicellular and multicellular components; there are no embryos free in the body cavity.

FIG. 26. Very immature daughter redia of *E. revolutum,* 0.25 by 0.06 mm, showing germinal mass and a number of free embryos, the smallest of which are about the same size as the smallest multicellular components of the germinal mass.

FIG. 27. Immature daughter redia of *E. revolutum,* about 0.42 mm. long, showing germinal mass attached in posterior end of body cavity.

FIG. 28. Posterior end of immature daughter redia of *E. revolutum,* 0.28 by 0.09 mm, showing complex germinal mass from side view; only one embryo present in body cavity.

FIG. 29. Posterior end of immature daughter redia of *E. revolutum,* 0.36 by 0.12 mm, showing complex germinal mass attached at posterior end of body cavity; largest cercarial embryo present is just in front of germinal mass.

FIG. 30. Posterior end of immature daughter redia of *E. revolutum,* 0.40 by 0.12 mm, showing complex germinal mass and a number of free embryos.

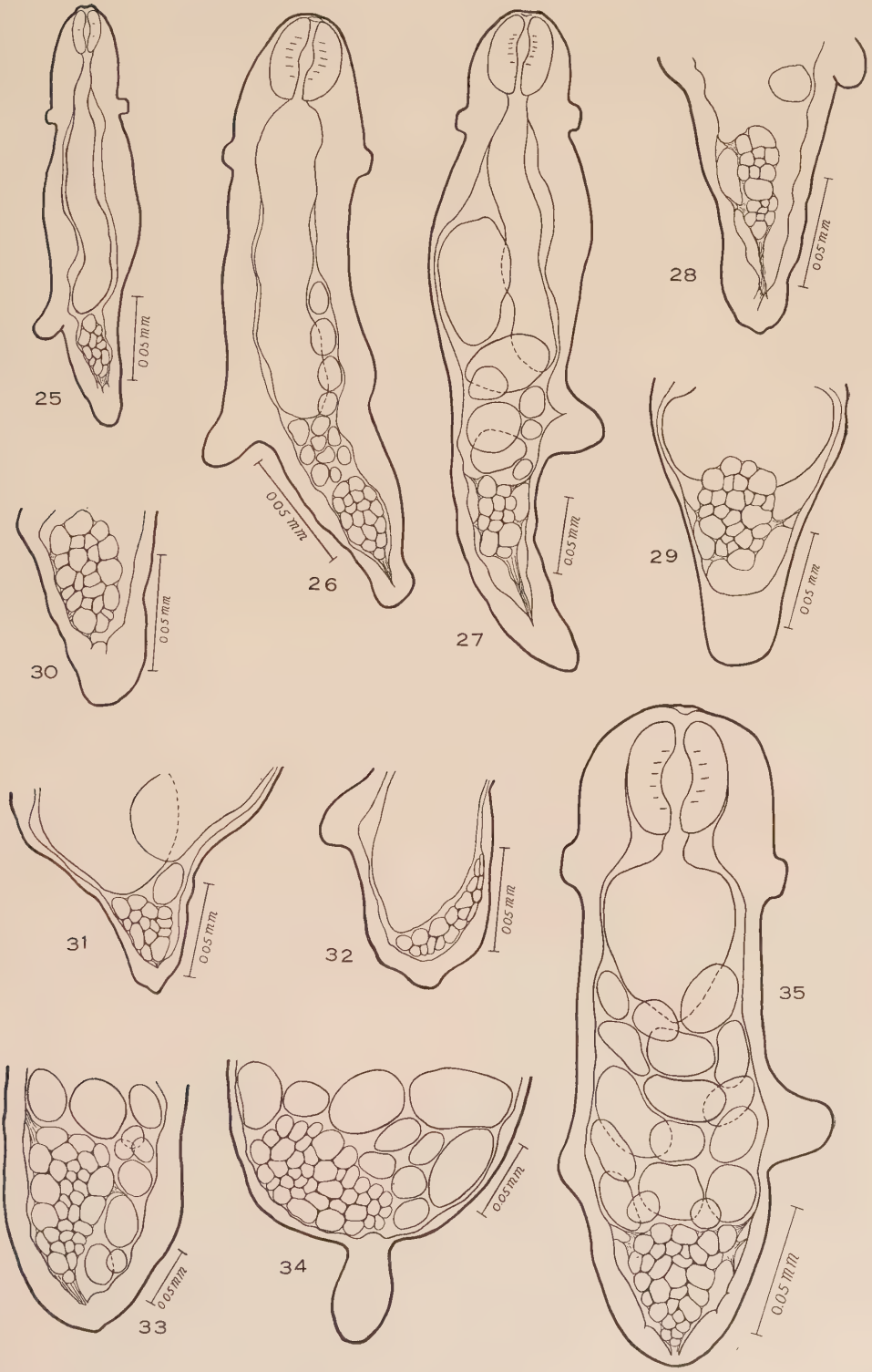
FIG. 31. Posterior end of almost mature redia of *E. revolutum,* 0.50 by 0.14 mm, showing germinal mass; body cavity is packed with large cercarial embryos none of which are mature.

FIG. 32. Posterior end of old redia of *E. revolutum,* 0.70 by 0.25 mm, with flattened germinal mass at one side of posterior end of body cavity; the anterior end of a cercaria is shown pressed against the germinal mass.

FIG. 33. Posterior end of a mature mother redia of an unidentified echinostome from *Physa* sp., about 0.98 mm long, showing large complex germinal mass attached at one side at the posterior end of the body cavity; numerous embryos including mature daughter redia are present; smallest free embryos are about the same size as the largest multicellular components of germinal mass.

FIG. 34. Posterior end of mature mother redia of an unidentified echinostome from *Physa* sp., showing large complex germinal mass.

FIG. 35. Daughter redial embryo of an unidentified echinostome from *Physa* sp., showing large complex germinal mass attached at the posterior end of the body cavity.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limited to the anterior end, and was curved around so that its posterior tip was frequently close up against the pharynx. Later we found one mature echinostome infection in which the daughter rediae had intestines exactly like those of these mother rediae. We, therefore, considered that they were of the same species, but were unable to make a specific identification.

The first infection with this form that was found contained seven mother rediae the smallest of which was 0.36 by 0.11 mm and the largest 1.50 by 0.30 mm. There were no free daughter rediae and none of the embryos even in the largest mother rediae appeared ready to escape. In the second infection only one mother redia was found. It was still immature but measured 1.20 by 0.30 mm. In the third infected snail seven mother rediae were counted. The smallest of which measured 0.52 by 0.12 mm and the largest 1.70 by 0.25 mm. The largest of these rediae contained 67 daughter redial embryos the largest of which, about 0.19 mm in length, did not appear ready to escape. Another of these mother rediae contained 41 embryos, of which three showed a differentiated pharynx and intestine. No free daughter rediae were present in this infection. The fourth infection with this species contained mature daughter rediae all about the same size. These rediae were about 1 mm or slightly more in length, and were crowded with embryos in various stages of development and mature cercariae which could be identified as echinostomes.

Plate IV

Description of Figures

Fig. 36. Posterior end of an unidentified immature echinostome mother redia from *Helisoma trivolvis,* about 0.72 mm long, showing germinal mass attached at posterior end of body cavity; numerous daughter redial embryos present, a few almost mature.

Fig. 37. Posterior end of mature mother redia of an unidentified echinostome from *H. trivolvis,* 0.80 by 0.25 mm, showing germinal mass attached at posterior end of body cavity; numerous redial embryos present, some fully developed; same species as Fig. 36.

Fig. 38. Immature daughter redia of unidentified echinostome from *Lymnaea stagnalis, appressa,* 0.50 by 0.05 mm, showing germinal mass composed of both unicellular and multicellular components attached at the posterior end of the body cavity.

Fig. 39. Posterior end of immature daughter redia of an unidentified echinostome from *Lymnaea stagnalis appressa,* 0.40 by 0.10 mm, from same infection as Fig. 38; shows complex germinal mass attached at the posterior end of the body cavity; numerous embryos present, the smallest of which are no larger than the largest multicellular components of the germinal mass.

Fig. 40. Posterior end of mature mother redia of *Petasiger nitidis,* 0.70 by 0.08 mm, showing complex germinal mass pushed against the wall of body cavity at one side by a well-developed daughter redial embryo.

Fig. 41. Immature daughter redia of *P. nitidis,* 0.30 by 0.04 mm, showing germinal mass attached at posterior end of body cavity and a few small free embryos; germinal mass appears to be composed of only unicellular components.

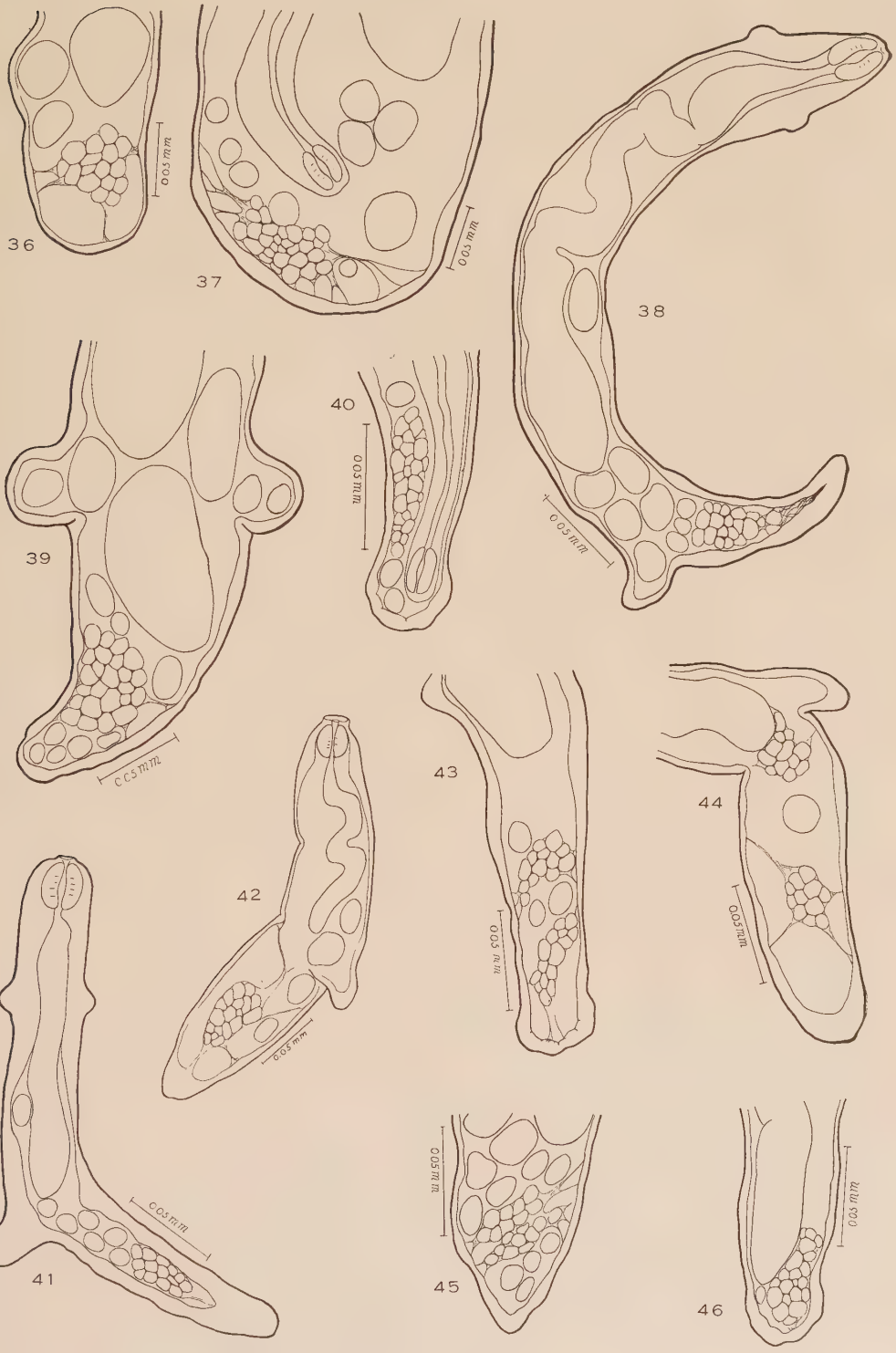
Fig. 42. Immature daughter redia of *P. nitidis,* about 0.30 mm in length, with complex germinal mass attached in posterior end of the body cavity and a few small free embryos.

Fig. 43. Posterior end of immature daughter redia of *P. nitidis,* 0.40 by 0.60 mm, showing germinal material in two masses attached to wall in posterior part of body cavity and a few small embryos.

Fig. 44. Posterior end of immature daughter redia of *P. nitidis,* about 0.41 mm in length, showing germinal material in two masses, one attached to wall near posterior end of body cavity and the other attached to posterior tip of intestine; only a few small free embryos present.

Fig. 45. Posterior end of immature daughter redia of *P. nitidis,* 0.60 by 0.12 mm, showing germinal mass attached to wall at posterior end of body cavity divided into two parts; numbers of embryos present, the largest measuring 0.13 by 0.05 mm.

Fig. 46. Mature daughter redia of *P. nitidis,* 1.5 by 0.15 mm, showing germinal mass attached at posterior end; large numbers of embryos of all sizes present; anterior end of cercarial embryo pushed against germinal mass.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45
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46
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The germinal masses were studied in all the mother rediae found. In the larger ones they were characteristically large, with many components both unicellular and multicellular, and were attached to one side of the posterior end (Figs. 33 and 34). In the two smallest mother rediae, which were 0.36 by 0.11 mm and 0.40 by 0.14 mm, the germinal masses were rounded and attached at the posterior tip of the body cavity. They contained numerous components both unicellular and multicellular. In these two rediae the embryos were numerous in the body cavity, but were all small "germ balls."

A few of the largest daughter redial embryos had broken out of the mothers and were studied. They had large germinal masses with both unicellular and multicellular components attached at the posterior end of the body cavity and a number of small free embryos (Fig. 35). The germinal masses were also studied in a few of the mature daughter rediae. They were like those of the mothers, being large with numerous components, both unicellular and multicellular, and were attached at one side of the posterior end.

The germinal masses of the rediae of this species were in general the largest and most complex of any seen in the rediae studied in the order FASCIOLATOIDEA. They seemed to be very active and persistent centers of multiplication organized for the production of rather large numbers of individuals.

6. Echinostome mother rediae from *Helisoma trivolvis* (Say). In the large series of specimens of *H. trivolvis* examined from the pond in Wilderness Park there were two which contained mother rediae only, one with three and the other with four. They were medium sized, about 0.7 to 0.8 mm in length, and appeared to be just about reaching maturity. The intestines extended for about three-fourths the body length. No free daughter rediae were found in either of these infections. While it is not possible to identify these rediae with certainty they appeared to be echinostomes and may have belonged to *E. revolutum,* since this species was very common in this snail in this pond. These mother rediae had germinal masses with both unicellular and multicellular components attached at the posterior end of the body cavity (Figs. 36 and 37).

7. Echinostome from *Lymnaea stagnalis appressa* (Say). One echinostome infection that seemed to be a different species from any of the others studied was found in a specimen of *Lymnaea stagnalis appressa.* This infection contained only daughter rediae of various stages of development. They were characterized by an unusually large intestine. In the smallest redia, 0.36 by 0.07 mm, there were no free embryos. A large germinal mass with both unicellular and multicellular components filled the posterior tip of its body cavity. The intestine extended back so that its posterior tip was practically in contact with the germinal mass.

Figures 38 and 39 show the germinal masses of two somewhat older immature rediae from this infection. In both of them the smallest free embryos are about the same size as the largest multicellular components of the germinal masses.

8. *Petasiger nitidis* Linton, 1928. Two mature infections of *P. nitidis,* the life cycle of which was worked out by Beaver (1939b) in the Douglas Lake region, were found in the specimens of *H. trivolvis* that were examined from the Wilderness Park pond during the summer. This species is easy to identify because of the characteristic large tail of the cercaria. In one of these infections a mature mother redia was found. Both snails contained immature as well as mature daughter rediae.

The single mother redia available for study had a size of 0.70 by 0.08 mm. It appeared to be mature since it contained two daughter rediae about ready to escape, one of which measured 0.30 by 0.03 mm. It had a large, well developed germinal mass attached at one side near the posterior end, which contained both unicellular and multicellular components (Fig. 40).

A series of immature daughter rediae was available for study. The youngest (Fig. 41) was only a little larger than the largest embryo within the mother redia. It had a little food in its intestine and a few embryos in the body cavity. Its germinal mass was attached at the posterior tip of the body cavity and appeared to be composed only of unicellular components. A slightly larger immature daughter redia is shown in Figure 42. The germinal mass in this case was clearly composed of unicellular and multicellular components. It was unusual in that it was separated from the wall of the body cavity and a little forward from its posterior tip. This suggests a very rapid growth in size of this redia in relation to the production of embryos. A series of somewhat larger immature rediae showed a rather peculiar relationship of the germinal mass for this species. In these forms there was also considerable space around the germinal masses not occupied by embryos. Stages like Figure 45 had a rather less compact germinal mass than those of the other echinostome rediae which was attached by a number of strands to the wall. In Figures 43 and 44 the loosely organized germinal mass appears in two parts, both with unicellular and multicellular components. It might be suggested that this separation of the germinal mass is associated with the especially rapid growth of the rediae of this species, as compared to the production of embryos. The anterior parts of these germinal masses, however, do not persist since in mature daughter rediae a single germinal mass was present in the posterior end as related forms (Fig. 46). The mature daughter rediae of *P. nitidis* are quite large and contain large numbers of cercariae and embryos. Three that were measured had lengths of 1.3, 1.5, and 1.8 mm respectively.

Discussion of ECHINOSTOMIDAE. The evidence is clear that in the echinostomes as in the psilostome, *P. ondatrae,* large germinal masses with both unicellular and multicellular components are present in both mother and daughter rediae attached at the posterior end of the body cavity. They appear to be persistent centers of multiplication of germinal cells, which make possible the production of the rather large numbers of individuals that are characteristically produced by members of this group. With one possible exception (Fig. 41) the germinal masses in the youngest rediae studied consisted of both unicellular and multicellular components. These germinal masses persist and appear still to be producing embryos in some very old rediae.

The mother rediae of this family appear to live for a long time and produce daughters continually because in most mature infections and even in some that had daughter rediae that appeared quite old, very immature daughter rediae were present. In the same way daughter rediae produce a continual stream of cercarial embryos over a long period of time, so that even in what appear to be very old rediae there will be present a few mature cercariae, embryos in all stages of development, and a germinal mass.

GENERAL DISCUSSION

Literature. The literature on the germ cell cycle, and the mechanism of reproduction in the germinal sacs or the order FASCIOLATOIDEA is very scanty and rather confusing. The only detailed study of the germ cell cycle in this group that was found was made on an echinostome species, *Parorchis acanthus,* by Rees (1940). This author traced the cells of the germinal line through all stages of the life cycle of this species and demonstrated that multiplication in the germinal sacs is by division of the original fertilized ovum without any reduction of the chromosomes by a maturation process. Only one first generation redia is present in the miracidium-mother sporocyst stage of this species, and the numbers of embryos produced in the rediae of both generations appear from her descriptions and figures to be quite limited. Also, it seems in this species that there is a rather synchronous development of embryos in both generations of rediae, since all those present appear to be about the same size. Finally, there is no indication of the presence of any germinal mass at the posterior tip of the body cavity in her description of the rediae, and she definitely stated that no "germ mass" is present. It would appear, therefore, that the germinal development in the rediae in *P. acanthus* differs from that found in the rediae of the echinostomes we studied in which the number of individuals produced by the rediae is much larger, and in which rather large persistent germinal masses are present in both mother and daughter rediae. The evidence does not seem to us to be sufficient to determine whether the simplified germ cell cycle of *P. acanthus* is a primitive condition for the echinostomes or a secondary modification related to a reduced production of individuals.

Apparently the only author who has described germinal masses for the FASCIOLATOIDEA is Brooks (1930, Figs. 44, 45, and 62). He described and figured "germ masses" in the posterior ends of rediae of an amphistome and two echinostome species. Since his studies were entirely from sections it is difficult to compare his findings directly with ours. He was, however, the first to point out the "germ mass" as a mechanism of reproduction in rediae.

We also found a few figures of rediae in the literature with structures depicted in the posterior ends of the body cavity that might possibly be interpreted as germinal masses (Looss, 1892, Figs. 7, 9, and 10; Looss, 1896, Figs. 128, 129, 130, 135, and 136; Cort, 1915, Figs. 1, 10, 11, 14, 15, and 25). Considering the prominence and characteristic structure of the germinal masses which we found in the rediae studied, especially in the psilostome and echinostome species, it is surprising that these structures have been almost completely overlooked. This is probably due to the neglect of the study of living material of rediae, especially in the immature stages.

Amphistomes. It might be suggested that in an early stage of the redial embryos of *A. parvum* a cell of the germinal line becomes localized just back of the primordium of the intestine and goes through a limited series of divisions to form the morula-like germinal mass that later becomes firmly attached at the posterior tip of the body cavity. Since there is evidence that the number of unicellular components (germinal cells) in the germinal mass in this species is reduced as embryos are separated from it, it seems probable that division of the germinal cells is limited to an early period, and that possibly all the embryos that

are produced are represented by components in the germinal mass by the time the first embryo is given off. At any rate, it seems clear that there is no adhesion of embryos to the germinal mass for any considerable time as multicellular components, and that the germinal mass is not a true persistent center of multiplication of germinal cells capable of producing large numbers of embryos. In this species, therefore, it is a mechanism that makes possible the production of only a limited number of embryos. It may be suggested as a working hypothesis that this simple type of germinal mass may be characteristic of species of the family Paramphistomidae in which only small numbers of individuals are produced. Observations on this group suggest in general a limited production of individuals in the germinal sacs. In specific cases the numbers may be very small. Bennett (1936) estimated that in *Cotylophoron cotylophorum* only about 225 cercariae were produced from a single fertilized ovum, and Takahashi (1928, cited from Bennett, 1936) gave the number for *Paramphistomum cervi* as only about 180. Certainly for such limited production of individuals no complicated germinal mechanism is needed. It may be, however, that further studies will show that there are species of amphistomes in which larger numbers of individuals are produced, and in which the germinal masses have become more persistent centers of multiplication of germinal cells than in *A. parvum.* If we accept the view stated earlier that the PARAMPHISTOMIDAE represent a very primitive and generalized group of the digenetic trematodes, this simple mechanism for reproduction found in the rediae of *A. parvum* becomes very significant in relation to the more complicated mechanisms in more specialized groups. It is in fact not difficult to understand how a germinal mass such as that of the echinostomes could be derived from it as an adaptation for the production of larger numbers of individuals. The development of such a germinal mass is really the result of the acquisition by the germinal cells of the ability to continue dividing throughout the life of the rediae.

Notocotylids. In our material of *N. urbanensis* only two mother rediae from one infection were available for study. In them were found large germinal masses containing both multicellular and unicellular components. This situation appears to be related to the fact that the mother rediae are produced in small numbers in *N. urbanensis* (6 to 8 being characteristic) and that each of them produces a considerable number of embryos. As noted above, counts, probably not complete, of daughter rediae of this species in six snails gave an average number of 289. Also, we gained the impression that the production of embryos by the mother rediae was much greater than by the daughters. On the other hand in the large numbers of daughter rediae studied, the germinal masses appear like these of *A. parvum* to consist only of unicellular components. Also, they did not appear to be persistent centers of multiplication of germinal cells, since sometimes even in rediae not yet mature the numbers of components in them are greatly reduced. It, therefore, can be concluded that the phase of division of the germinal cells in the daughter rediae of *N. urbanensis* is limited to an early period of development as in the rediae of *A. parvum,* producing a definite limitation on the numbers of cercarial embryos produced.

Of course we can hardly conclude on the basis of the study of just one species, that the relations we found of germinal masses in the mother and daughter rediae of *N. urbanensis* are characteristic for the family NOTOCOTYLIDAE. However, it is

interesting to note in this connection that Herber (1942, Figs. 3, 4, 17 and 18) described and figured the mother rediae of two other species of notocotylids as much larger than the daughters and containing much larger numbers of embryos. Mature daughter rediae of species of this family have been described and figured by a number of workers. They, in general, contain small numbers of embryos and only a few cercariae (about 3 to 5) far enough advanced to show eyespots. It becomes a matter, therefore, of considerable interest to study the germinal mechanism in the mother and daughter rediae of other species of this family.

Echinostomata. In the psilostome and echinostome species studied the same general mechanism for the multiplication of the germinal cells was found in both mother and daughter rediae. Germinal masses consisting of both unicellular and multicellular components are present attached at the posterior end of the body cavity. Multicellular components are present in the germinal masses even in very young or even embryo rediae. These germinal masses appear to persist for practically the whole life of the rediae, and to give off a constant stream of embryos. This is indicated by the fact that germinal masses and the smallest free embryos are still present in very old rediae. The germinal masses in this group, therefore, appear to be persistent centers of multiplication of germinal cells, adapted for the production of considerable numbers of embryos over a rather long period of time.

Unfortunately, we did not make a series of studies of redial embryos at different stages to follow the development of the germinal masses in any of the species of this group. At any rate, we can suggest that at an earlier stage than any we have shown, the germinal masses of the rediae of these species would consist of a group of unicellular components similar to those of the rediae of the amphistome. In fact, in one case in a young daughter redia the germinal mass seemed to be composed only of unicellular components. However, unlike the germinal cells in the mother and daughter rediae of *A. parvum,* and those of the daughter rediae of *N. urbanensis,* the germinal cells in the germinal masses of the psilostome and echinostome rediae that were studied have the potentiality of continuing to divide over a long period of time. Some of them, however, even in very early stages start embryonic development and remain attached to the others for their early stages as multicellular components of the germinal mass. This produces the characteristic structure of the germinal masses found in the rediae of this group, which in some cases are large with considerable numbers of both unicellular and multicellular components. During the reproductive life of the rediae there is a gradual breaking off of multicellular components and a continued division of the unicellular components to form new germinal cells some of which then develop into embryos to replace those that have broken away. This accounts for the persistence of the germinal masses, and makes possible the constant replacement of the embryos which have completed their development in the rediae and escaped from them. This mechanism in the mother rediae, therefore, makes possible the production of sufficient numbers of daughter rediae to fill the digestive gland of the infected snail. It also makes possible the production of a considerable number of cercariae in the daughter rediae which escape in rather small numbers each day over the whole mature life of the infection. This produces in these psilostome and echinostome species a much larger number of individuals than are produced by the restricted division of germinal cells of the

germinal masses in the mother and daughter rediae of the amphistome, *A. parvum,* and in the daughter rediae of the notocotylid, *N. urbanensis.*

From our own studies there is considerable evidence that the numbers of individuals produced by the rediae of the psilostome and echinostome species are much greater than the numbers produced by the amphistome and notocotylid. In some cases the number of embryos counted in a single mature redia was close to or surpassed a hundred. Also, the numbers of daughter rediae counted in individual infections was surprisingly large. Especially striking were the counts of infections in large specimens of *H. trivolvis* which ran from over 500 to almost 4,000. These counts of daughter rediae are especially significant since the numbers of mother rediae produced by the small mother sporocysts of this group are, as far as known, characteristically small.

Of special significance are the data which give comparisons between the numbers of daughter rediae of the same species in small and large snails. An example of this relation was given above for counts of the daughter rediae of *E. revolutum.* In nine specimens of *H. antrosum percarinatum* with a shell diameter of from 10 to 12 mm the counts varied from 175 to 540 with an average of 360, while in seven large specimens of *H. trivolvis,* about 25 mm in diameter, the counts of daughter rediae varied from 558 to 3,960 with an average of 1,724. These counts suggest that the number of daughter rediae in an infection with this species is determined primarily by the space and food available in the digestive gland of the infected snail rather than by the reproductive potential of the mother rediae.

It is interesting in this connection that Wisniewski (1937) found this same relation in production of indivduals in *Parafasciolopsis fasciolaemorpha* Ejsmont in *Coretus corneus.* This trematode belongs to the FASCIOLIDAE and thus falls into Szidat's suborder ECHINOSTOMATA. In Table 6 of this paper we find that the number of daughter rediae counted is definitely related to the size of the snails. In five snails 12 to 16 mm in diameter the numbers varied from 670 to 1800 with an average of 1058, while in six larger snails which varied from 22 to 32 mm in diameter, the counts varied from 1700 to 8000 with an average of 4217.

Estimates of total numbers of cercariae that can be produced from a single fertilized ovum in this group are at best rough guesses. Cort (1944) hazarded the guess that in certain echinostomes the number of cercariae produced might be well over 25,000. In the light of our present studies this seems a conservative figure. In infections containing 500 daughter rediae it would need only the production of 100 cercariae during the whole life of each redia to bring this figure to 50,000. Cort (1922), also, found that for an echinostome species on which counts were made, from a few hundred to nearly a thousand cercariae escaped in a 24-hour period. More complete counts over much longer periods were made by Wisniewski (1937) for *Parafasciolopsis fasciolaemorpha,* which ranged from 12,000 to 100,000 in different infected snails.

It can be suggested, therefore, that in the order FASCIOLATOIDEA in which, as far as is known, the mother sporocysts produce only a very few individuals, there has developed in the rediae in certain species, at least, a mechanism for the production of greatly increased numbers of individuals in the form of a germinal mass which is a persistent center of multiplication of germinal cells.

These germinal masses found attached at the posterior end of the body cavity of

the rediae of the psilostome and echinostome species can be compared directly with the germinal masses reported earlier in the daughter sporocysts of plagiorchiids (Cort and Olivier, 1943; Cort and Ameel, 1944). In the plagiorchiids there is, also, only one germinal mass in each secondary germinal sac. However, it is not attached but floats freely among the developing embryos. In general the germinal masses in the plagiorchiid daughter sporocysts appear to be larger and to have larger numbers of components than those of the rediae of the ECHINOSTOMATA. In the plagiorchiids, also, they are interpreted as persistent centers of multiplication of the germinal cells which are found still producing embryos even in the oldest infections. As far as we have any direct evidence (cf. Cort, 1922; Dubois, 1929) and from general observation the conclusion can be drawn that the production of cercariae in the plagiorchiids is very much greater than in the ECHINOSTOMATA. This is probably due mostly to a more rapid and perhaps more persistent production of embryos in the daughter sporocysts by their larger germinal masses. Also, in some cases there is a greater production of secondary germinal sacs by the extensive division of the germinal cells that takes place in the complicated mother sporocysts of this group.

SUMMARY

The germinal material was studied in rediae from natural infections of species of PARAMPHISTOMIDAE, NOTOCOTYLIDAE, PSILOSTOMIDAE, and ECHINOSTOMIDAE, which belong to the order FASCIOLATOIDEA Szidat, 1936. In an amphistome, *Allassostoma parvum* Stunkard, 1916, each immature mother and daughter redia has attached at the posterior end of the body a simple morula-like germinal mass composed of unicellular components which are considered to be germinal cells. Evidence from the study of older rediae indicates that these simple germinal masses are gradually used up by the separation of their components to form embryos. In the daughter rediae of a notocotylid, *Notocotylus urbanensis* (Cort, 1914) the germinal masses are similar to those of *A. parvum*. In this case, also, the number of the components is reduced as the embryos are separated. It can be suggested that this simple type of germinal mass is produced by a limited division of germinal cells early in the development of the rediae, and can result in the production of only a limited number of embryos. In two mature mother rediae of *N. urbanensis* the germinal masses were larger and were composed of both unicellular and multicellular components. This suggests a more extended period of multiplication of the germinal cells associated with a greater production of individuals.

Studies were also made of the germinal material in the rediae of a psilostome, *Psilostomum ondatrae* Price, 1931, and five echinostome species, *Echinostoma revolutum* (Froelich, 1802), *Petasiger nitidus* Linton, 1928, and three that were not identified. In mother and daughter rediae of these species, both the immature and mature, there were found rather large germinal masses, composed of both unicellular and multicellular components, which were attached at the posterior end of the body cavity. They were still present in the very oldest rediae examined. They resemble in structure the floating germinal masses of the daughter sporocysts of the plagiorchiids. They are interpreted as persistent centers of multiplication of germinal cells, and are considered as a mechanism for the production of rather large numbers of embryos. Evidence was presented on the greater production of individuals in the psilostome and echinostome rediae, as compared with those of amphistomes and notocotylids.

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A NOTE ON THE NEMATODE GENUS *PSEUDOPHYSALOPTERA*

The nematode species *Physaloptera formosana* has apparently never been reported since it was originally described by Yokogawa in 1922 (Trans. Jap. Path. Soc. **12**: 201) from a species of *Sorex*. Its taxonomic status has been in question, partly because of the incomplete description which accompanied the naming of the species. Morgan (1941, The Physalopterinae (Nematoda) of North American Vertebrates, Thesis, University of Wisconsin), lists the species as "Physalopterinae inadequately described" and states that it should probably be transferred to the genus *Pseudophysaloptera* Baylis 1934. Morgan later (1946, Trans. Wis. Acad. Sci., Arts and Letters, **38**: 273–292) again emphasized the inadequacy of the original description.

Review of the present status of *Pseudophysaloptera* in connection with the study of a new species of the genus (Lincicome, 1948, in press) convinces the writer that enough information is contained in Yokogawa's original description to warrant placing *P. formosana* in the genus *Pseudophysaloptera*.

The characters upon which Baylis recognized *Pseudophysaloptera* are wholly those of the male organism. The most evident difference between *Pseudophysaloptera* and *Physaloptera* concerns the caudal papillae which are all sessile in the former and pedunculated in part in the latter. Yokogawa clearly stated "In the young male, there are a pair of sessile pre-anal papillae, a little bigger pre-anal papillae unpaired, and three pairs of post-anal sessile papillae" and commented further that "It is, however, difficult to detect these papillae in the adult male." This seems to give sufficient evidence that the structure of the male caudal papillae is that of the genus *Pseudophysaloptera*, and, on this basis, it is proposed to transfer *Physaloptera formosana* to the genus *Pseudophysaloptera*. The emended name of this organism then becomes *Pseudophysaloptera formosana* (Yokogawa, 1922).

With the inclusion of *P. formosana* the genus *Pseudophysaloptera* contains three species: (1) *P. soricina* Baylis, 1934; (2) *P. riukiuana* Lincicome, 1948; (3) *P. formosana* (Yokogawa, 1922). The latter may be distinguished from other members of the genus by the number and arrangement of caudal papillae in the male. The caudal papillae formula for *P. formosana* is: 1 pair pre-anal; 1 single pre-anal; and 3 pair post-anal. Baylis 1934 records only four pair of post-anals for *P. soricina* while Chen (1937) as well as Crusz (1946) both indicate the papillae are numerous. Lincicome (1948) records two pre-anal pair, a single pre-anal and six post-anal pairs for *P. riukiuana*.

TABLE 1.—*Host-parasite list of members of the genus* Pseudophysaloptera

Species	Host	Locality	Reference
P. soricina	*Crocidura* sp.	Tanganyika Territory, Africa	Baylis, 1934, Ann. Mag. Nat. Hist. Ser. 10, *13:* 338–353
"	*Suncus coerulus*	China	Chen, 1937, Parasit. *29:* 419–434
"	*Sorex p. personatus* *Sroex f. fumeus*	U. S. A.	Morgan, 1941, Thesis University of Wisconsin
"	*Blarina b. brevicauda* *Suncus coeruleus kandianus*	Ceylon	Baylis, 1944, Ann. Mag. Nat. Hist. Ser. 11, *11:* 793–804
"	*Suncus caeruleus*	Ceylon	Crusz, 1946, Ceylon J. Sci. Sect. B, *23:* 47–66
P. riukiuana	*Suncus murinus*	Okinawa, Ryukyu Islands	Lincicome, 1948, Pacific Science, (In Press)
P. formosana	*Sorex* sp.	Formosa	Yokogawa, 1922, Trans. Jap. Path. Soc. *12:* 201

Table 1 presents hosts and localities from which members of *Pseudophysaloptera* have been recorded.—DAVID RICHARD LINCICOME, *School of Medicine, University of Wisconsin, Madison.*

INVASION ROUTE OF *NOSEMA* SP. IN THE POTATO TUBERWORM, AS DETERMINED BY LIGATURING

A microsporidian infection, caused by an unidentified species of *Nosema*, has occurred in several insectaries in which the potato tuberworm, *Gnorimoschema operculella* (Zell.), is being reared. This insect is being used as a host in the propagation of *Macrocentrus ancylivorus* Roh., an important hymenopterous parasite of the oriental fruit moth, *Grapholitha molesta*

(Busck.). During the course of studies on the life history of the protozoan, it became desirable to ascertain through which part of the alimentary tract the organism was able to penetrate prior to its extensive development in the tissues of the host's body cavity. That the microsporidian may pass from one generation to the next by way of the host egg is already known (Allen and Brunson, 1947, Science, *105,* 394). The invasion route by way of the alimentary tract of the larva has been assumed to be through the wall of the midgut. The purpose of the present note is to report confirmation of this assumption by means of a ligaturing technique.

Healthy tuberworm larvae of the fourth instar were used as test animals. After being permitted to feed for 24 hours on a potato tuber previously sprayed with an aqueous suspension of *Nosema* spores, ligatures of fine silk thread were applied at various points along the body of the larvae which were then placed in glass rearing chambers. Four days later smears were made of the blood taken from the body cavity of each insect both anterior and posterior to the ligature. The Giemsa-stained blood smears were then examined microscopically for the presence or absence of *Nosema* spores. Successfully ligatured larvae ordinarily survived for about five days. If the ligature were not tightly applied, or if the larva were injured during the manipulations, the ligatured insect turned black (especially the anterior portion), became motionless, and died quickly.

A total of 49 examinations were made with uniform results being obtained. These results are summarized in Table 1.

TABLE 1.—*Presence or absence of* Nosema *spores in infected and ligatured potato tuberworms*

Location of ligature	Presence or absence of *Nosema* spores	
	Anterior to ligature	Posterior to ligature
Posterior end of third thoracic segment	−	+
Posterior end of first abdominal segment	+	+
Posterior end of fifth abdominal segment ...	+	+
Posterior end of sixth abdominal segment ..	+	−

Upon dissection of a tuberworm larva, the foregut is found to end at the posterior end of the third thoracic segment. The midgut ends at the middle of the sixth abdominal segment where the paired Malpighian tubes originate and extend anteriorly to the first abdominal segment and then coil back.

From the results given in Table 1, it may be concluded that the microsporidian is incapable of invading the body cavity of the tuberworm through the wall of either the foregut or that of the hindgut. On the other hand, invasion through the wall of the midgut (and possibly through the Malpighian tubes) takes place regularly.

Entirely comparable results were obtained in a series of 15 experiments in which the larvae were ligatured prior to being permitted to feed on spore-contaminated potato.

The same ligaturing technique here described has been similarly used in determining the invasion route of the virus of a polyhedrosis of the California oakworm, *Phryganidia californica* Pack. The results obtained also suggested the wall of the midgut as the route of invasion of the virus into the body cavity. This was concluded from the fact that, 5 days after ligaturing, polyhedral bodies were found in the body cavity posterior to the ligature when the latter was placed at the posterior end of the second thoracic segment and anterior to the ligature when it was placed at the posterior end of the fifth abdominal segment. This area was found by dissection to harbor the midgut of the larva.—ALBERT ABDEL-MALEK AND EDWARD A. STEINHAUS, *Laboratory of Insect Pathology, Division of Biological Control, University of California, Berkeley, California.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Preliminary Announcement of the Twenty-Third Annual Meeting

Sunday, Monday, Tuesday, and Wednesday, December 5 to 8, 1948
New Orleans, Louisiana

Following the decision of the Council and action taken by the Society at the Chicago (1947) meeting, the American Society of Parasitologists will convene for a three-day meeting in conjunction with the convention of the American Society of Tropical Medicine, the National Malaria Society, and the American Academy of Tropical Medicine in New Orleans, Louisiana. The Roosevelt Hotel will be the official headquarters for the Society and all functions will be held at that Hotel.

Program

Dec. 5 (Sunday):	Registration, Roosevelt Hotel, Headquarters.
Dec. 6 (Monday):	9:00–12:00 noon—Regular session.
	2:00– 5:00 p.m.—Regular session.
	7:00 p.m.—Dinner and Council meeting.
Dec. 7 (Tuesday):	9:00–12:00 noon—Joint session with National Malaria Society and American Society of Tropical Medicine.
	2:00– 4:30 p.m.—Panel discussion, "Arthropod Vectors of Human Disease," Dr. Cornelius Philip, Vice-President, presiding.
Dec. 8 (Wednesday):	9:00–11:00 a.m.—Regular session.
	11:00 a.m.—Presidential Address, "Reflections of a Medical Parasitologist," Dr. E. C. Faust, President.
	12:30– 1:30 p.m.—Annual luncheon and business session.
	2:30– 5:30 p.m.—Laboratory meeting and tea.

All members are urged to attend this convention as it appears to be an unusual opportunity to meet members not only of the American Society of Parasitologists but also of our sister societies, the American Society of Tropical Medicine, the National Malaria Society, and the American Academy of Tropical Medicine.

Respectfully,
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